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The Role of Three-dimensional Ultrasound in the Diagnosis and Treatment of Uterine Pathology

Mavrellos, Dimitrios

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The Role of Three-dimensional Ultrasound in the Diagnosis and Treatment of Uterine Pathology

MD thesis

Dr Dimitrios Mavrelos

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List of abbreviations

2D – two – dimensional

2D – SIS – two dimensional saline infusion sonohysterography

3D – three – dimensional

3D – PDA – three – dimensional power Doppler angiography

3D – SIS – three – dimensional saline infusion sonohysterography

AUC – area under the curve

B- mode – brightness mode

BMI – body mass index

bFGF – basic fibroblast growth factor

CI – confidence interval

CT – computed tomography

CW – continuous wave Doppler

D&C – dilatation and curettage

EGF – epidermal growth factor

EIC - Endometrial intraepithelial carcinoma

EPAGU – Early Pregnancy & Acute Gynaecology Unit

FI – flow index

FIGO – International Federation of Gynaecology and Obstetrics

GnRH - Gonadotrophin releasing hormone

GDTU – Gynaecologic Diagnostic & Treatment Unit

HRT – hormone replacement therapy

ICC – interclass correlation coefficient

IQR – interquartile range

KCH – King’s College Hospital

MRI – magnetic resonance imaging

OR – odds ratio

PI – pulsatility index

PFR – pulse repetition frequency

PW – pulsed wave Doppler

RADAR – radio detection and ranging

RR – relative risk

ROC – receiver operating characteristic curve

SD – standard deviation

SE - standard error

SIS – saline infusion sonohysterography

SONAR – Sound navigation and ranging

TCRF – transcervical resection of fibroid

TGF – b - transforming growth factor beta

TGF - $\beta 3$ - tumour growth factor – $\beta 3$

UCH – University College Hospital

VEGF - vascular endothelial growth factor

VFI – vascularisation - flow index

VI – vascularisation index

VOCAL - virtual organ computer aided analysis

WMF – wall motion filter

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Abstract

This thesis investigated the role of three – dimensional transvaginal ultrasound in the identification, classification and management of uterine pathology. We performed serial measurements of fibroids over a period of time to examine their natural history. We found that fibroids tend to grow at a relatively fast average rate in pre-menopausal women, which is influenced by the initial fibroid volume and patients' demographic characteristics. Our findings could help to rationalise the follow up and plan better the treatment of women with fibroids. Our results also provided novel insights into the possible pathogenesis of fibroids. 3D – SIS has been previously demonstrated to be equivalent to hysteroscopy to measure the degree of submucous fibroids protrusion into the endometrial cavity. However compared to hysteroscopy, 3D – SIS provides additional information including objective measurement of fibroid size and position. We evaluated these additional ultrasonic variables and identified diameter and size of the intramural portion as predictive of complete resection at a single TCRF. GnRH analogues have been given to women with submucous fibroids before transcervical resection to improve the chance of complete resection. However evidence for this practice is limited and the treatment is associated with significant side effects. We carried out a double - blind , placebo – controlled, randomized trial which did not demonstrate a benefit in the preoperative administration of GnRH analogues in women scheduled for TCRF.

This thesis also investigated the role of 3D ultrasound in the diagnosis and treatment of endometrial cancer. Currently women with postmenopausal bleeding are investigated by transvaginal ultrasound to measure endometrial thickness. This results in a substantial

number of women needing endometrial biopsy to confirm benignity. We evaluated the ability of three 3D - PDA with objective quantification of vascularity indices to differentiate between benign and malignant lesions in women at high risk of endometrial cancer. We found that this shows promise but does not eliminate the need for biopsy. Currently women diagnosed with cancer undergo surgical staging that increases surgical morbidity. We investigated the usefulness of endometrial volume measurement in such patients to predict cancer stage which may be used in preoperative planning.

Statement of originality and personal contribution to work

I was personally involved in the design of all studies, application for Ethics approval and recruitment of all patients. I performed all ultrasound examinations, collected all data and performed all statistical analyses and interpretation of results, with the advice and guidance of those mentioned in the following section.

Acknowledgements

None of this work would have been possible without the inspiration and support of Mr Davor Jurkovic. Apart from teaching me how to be a better doctor he has taught me how to be a better person.

I would also like to thank my Research Fellow colleagues at King's College and University College Hospital, Dr Emma Sawyer, Dr Samir Helmy, Mr Joseph Yazbek, Miss Jara Ben – Nagi and Dr Tom Holland for their help and ideas.

In life I have been fortunate to enjoy the support and care of my parents, Dr Konstantine Mavrelou and Dr Katerina Mavrelou. I am grateful.

Evie, I love you.

Hypothesis

This thesis will investigate the following:

1. Uterine fibroids are slow growing tumours with a uniform growth rate in premenopausal women.
2. Submucous fibroids that can be resected completely by a single transcervical resection differ in their size, location and numbers compared to those who cannot be completely removed.
3. Women that receive GnRH analogues before transcervical resection of a submucous fibroid have improved probability of complete resection compared to those who receive placebo.
4. The endometrium in women with endometrial cancer has different ultrasonic and Doppler characteristics compared to women with benign lesions.
5. There is a positive correlation between the size of endometrial cancer and stage of disease.

Aims

The aims of this thesis are

- Investigate the natural history of fibroids and identify factors that may influence their growth.
- Assess the ability of 3D - SIS to evaluate submucous fibroids preoperatively and identify the characteristics, which may be useful to estimate the likelihood of a complete removal at transcervical resection.
- Determine whether preoperative administration of GnRH analogues for women scheduled to undergo TCRF improves the success of resection.
- Assess the ability of novel 3D ultrasound vascularity markers to discriminate between women with endometrial cancer and those with benign pathology in a high risk population.
- Assess the relationship between endometrial volume as determined by 3D ultrasound and stage of endometrial cancer at surgery.

Part I Background

[1] Technical aspects of ultrasound

1.1 Introduction

The female reproductive organs are concealed in the pelvis so that obstetrician gynaecologists need to employ a variety of techniques to assess their health. Starting from simple bimanual

examination and palpation and progressing to instruments such as Cuscoe's and Sims' speculum gynaecologists have always been interested to employ the latest technological developments to improve their diagnostic confidence. It is not surprising therefore that one of the first clinical applications of ultrasound, a diagnostic modality that is now in widespread use, was in the diagnosis of pelvic masses in women by Ian Donald in 1958 (Figure 1) (Donald et al. 1958). The ideal diagnostic test should be minimally invasive, highly sensitive and specific so that all patients with a disease are correctly identified without delay and healthy patients are reassured that their symptoms and not caused by a gynaecological abnormality. 2D



Figure 1 Excerpt from one of the initial scientific studies on the diagnostic uses of ultrasound

transvaginal ultrasound with high frequency probes has revolutionised gynaecological

practice and in many respects has vastly improved the diagnostic options of gynaecologists. In the not too far off future routine gynaecological examination will be mainly based on ultrasound and bimanual examination will be used only selectively. However there are still areas in the diagnosis of benign and malignant disease of the uterus that our diagnostic performance requires improvement. The purpose of this thesis is to evaluate whether three – dimensional ultrasound and the various modalities associated with it can improve the diagnosis of uterine pathologies and optimize their subsequent treatment.

1.2 *Physics and technical aspects*

Medical ultrasound was developed from metallurgical testing and RADAR/SONAR technologies. It is based on the principle of using a piezoelectric element to convert electrical current into ultrasound and transmitting this ultrasound through the medium of interest. The reflected ultrasound waves are then received by the same piezoelectric element and converted into electrical current and subsequently real time images.

1.2.1 Frequency, wavelength, phase of a wave

Waves are disturbances with a regular repeating pattern that travel across a medium. Sound waves are longitudinal, as opposed to transverse, in that the direction of wave travel is the same as the direction of energy transfer. The properties of any wave can be described by the frequency, wavelength and phase of the wave. Frequency (f) refers to the number of oscillations passing an observer in a unit of time usually measured in Hz (1 Hz = 1 cycle per second). Ultrasound refers to sound waves whose frequency falls outside the audible range i.e. higher than 20 kHz. The wavelength (λ) refers to the distance between two consecutive peaks or troughs of a wave. It follows that the speed of a wave is given by $c = f \times \lambda$. Phase

refers to the point in the wave that we take a given observation and is measured in degrees ($^{\circ}$) (Martin et al. 2003).

1.2.2 Ultrasound wave pressure, velocity and impedance

Ultrasound waves passing through a medium transport energy in the direction of wave travel. Through the cycles of the wave areas of compression and rarefaction are created in the medium. Excess pressure (p) refers to the change in pressure created in a point of the medium by the passage of the wave. The maximum excess pressure created by the wave is called amplitude of the wave. The amount of energy transported by the wave through a unit area presented at 90° to the direction of propagation is called the intensity of the wave (I). Logically, the intensity of the wave is proportional to the pressure amplitude of the wave ($I \propto p^2$).

The particles of different media respond differently when a wave of given pressure is applied to them. The response of each medium is called impedance (z) and it depends on the density (ρ) and stiffness (k) of the particular medium ($z = \sqrt{\rho k}$) measured in $\text{kg m}^{-1} \text{ s}^{-1}$. The impedance of each medium is given by $z = p/v$ where p is the local pressure and v the velocity of the wave in the medium (Kane 2009).

1.2.3 Reflection

When a sound wave travels across the interface of two media with different impedances (z_1 , z_2) the local pressure and particle velocity close to the interface must be the same across the two media. However the difference in acoustic impedance between the two media implies an abrupt change in pressure which is accommodated by the creation of a new wave that travels

back into medium 1, the reflected wave. The sums of pressures and velocities of the incident and reflected wave in medium 1 are then equal to the pressure and velocity of the wave in medium 2. The ratio of reflected to incident pressure is called the amplitude reflection coefficient (R_A) and this determines the amplitude of echoes arising at the boundaries between tissues with different impedances. As the intensity of a wave is proportional to the square of wave pressure amplitude and the energy transferred across a medium must remain constant it follows that the sum of incident and reflected wave intensities will be equal to the intensity of the wave in medium 2 ($I_t = I_i - I_r$). The ratio of reflected wave to incident wave intensity is called R_i while the proportion of wave that is transmitted is called the transmission coefficient ($T_i = 1 - R_i$) (Martin et al. 2003; Kane 2009).

1.2.4 Reflection coefficient (R_A)

The reflection coefficient between interfaces is an important property because it determines the amplitude of pressure that propagates beyond the interface and therefore the amount of echoes and so the amount of information that can be obtained beyond a given interface. For example the reflection coefficient between the liver and kidney is 0.006 so echoes from deeper structures can be obtained. In contrast the reflection coefficient between soft tissue and air is 0.999 and so no further useful echoes can be obtained beyond such an interface.

1.2.5 Scattering

When ultrasound waves encounter targets that are small reflection as described above does not occur. Instead the wave is scattered over a large range of angles. In fact, if the target is considerably smaller than the wavelength of the wave then scattering occurs uniformly over all angles. In addition the total ultrasound power scattered by small targets is much smaller

than that reflected at large interfaces. This is the reason why organ parenchyma, which contain a large number of small targets from the cellular to the macroscopic, produce a weak echo levels compared to organ interfaces.

1.2.6 Attenuation and absorption

As an ultrasound wave travels through tissues some of the wave's intensity is lost. When an ultrasound wave passes through a tissue the tissue particles vibrate in response to the pressure changes induced. Some of the vibration however is not passed on to the next particle in the chain but rather is retained as heat in the tissue. The heat is then dissipated and some of the wave's intensity is attenuated. In tissues this effect is dependent on the wave's frequency with higher absorption and therefore attenuation at higher frequencies. Clearly when high frequencies (10 – 15 MHz) are used the imaging of deeper structures becomes problematic as the degree of attenuation increases and the echoes obtained become very weak. Nevertheless higher frequencies are associated with shorter wavelengths and this higher resolution as the degree of scattering is reduced (Martin et al. 2003).

1.3 *Image formation*

1.3.1 Transducers

The device that transforms electrical transmissions to ultrasound pulses and also ultrasound echoes into electrical signals is the transducer. This is composed of a piezoelectric element whose property is to expand or contract when a voltage is applied across it and also to generate voltages when stretched or compressed. The commonest material used in medical transducers is lead zirconate titanate (Kane 2009). Because the characteristic acoustic

impedance of the piezoelectric element is 20 times higher than soft tissue a backing layer and a matching layer are required. The backing layer reduces the amount of ultrasound waves lost through reflection at the transducer/tissue interface whilst the impedance matching layer increases the amount of ultrasound waves transmitted at the transducer/tissue interface. The final element of the transducer is a lens which focuses the ultrasound beam. A variety of probes exist depending on the arrangement of transducers within them. The simplest arrangement is a linear array but other formats exist, such as a curvilinear, trapezoidal or sector probes. Transvaginal probes are usually of the sector type.

1.3.2 B – Mode image formation

A B – mode image is a cross – sectional image representing tissues and organ boundaries within the body. The image is constructed from echoes generated by the reflection of ultrasound waves at tissue boundaries (Figure 2). Each echo is represented as a point in the image and the amplitude of the echo determines the brightness of each point (Martin 2003). The ultrasound transducer is composed of up to 256 piezoelectric elements which are activated in sequence to emit an ultrasound pulse. After emission the transducer listens for returning echoes thus completing pulse – sequence which corresponds to a specific scan line. The next piezoelectric element in the sequence is then activated and so on until a sweep of all the elements is completed. Each 2D B – mode image is composed by a large number of such scan lines (≈ 100) each of which represents a pulse – echo sequence. In contrast to CT or MRI imaging a complete sweep of the pulse – echo sequence may take $1/30^{\text{th}}$ of a second allowing us to perform real time scanning (Whittingham 2003).

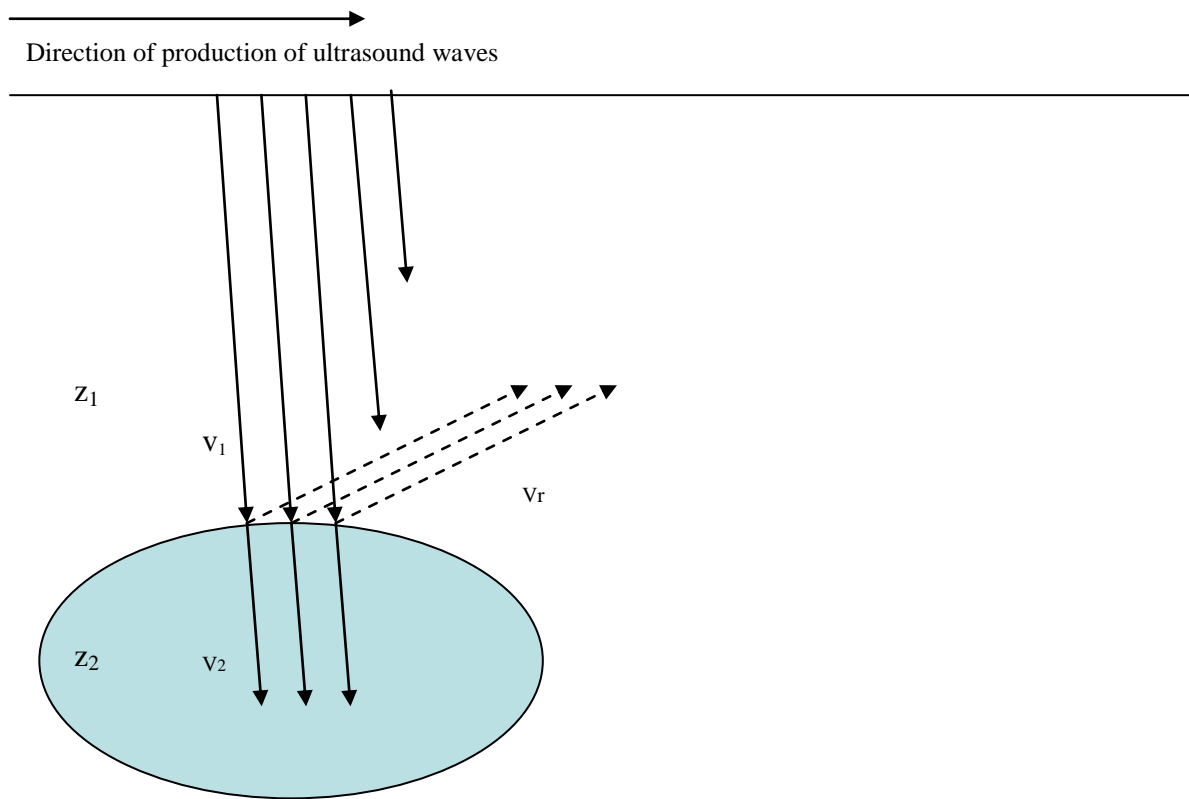


Figure 2 Schematic representation of the formation of B - mode images. The incident wave travelling with velocity v_1 in a medium of impedance z_1 encounters a new medium with impedance z_2 . A reflected wave with velocity v_r is produced so that $v_1 + v_r = v_2$. The wave then continues with velocity v_2 in the new medium.

1.3.2.1 Signal processing

Echoes received by the piezoelectric element are too weak to be displayed directly after reception and so need to be amplified. The first step is to amplify all returning echoes by the same factor, this being represented by the overall gain on an ultrasound machine. Because of tissue attenuation echoes returning from deeper tissues are weaker than those from more superficial interfaces. In order to achieve the aim of B – mode imaging, i.e. to relate

brightness on the screen to the strength of returning echoes, echoes that take longer to arrive are amplified more to counteract the effect of tissue attenuation. Returning echoes are then compressed and digitised so that the analogue signal received by the transducer is converted to a binary number representing the signal amplitude. In order to correctly display a B-mode image, apart from echo amplitude and the position of the scan line relative to other returning echoes, it is necessary to know the range of the echo to be displayed.

1.3.2.2 *Echo ranging*

It is possible to measure the distance of a target that has given rise to a specific echo if we know the speed of sound in the medium of interest as well as the time for the echo of the transmitted ultrasound pulse to return to the transducer. This relationship is described by $t = 2d/c$ (1) where t is the total time from the transmission of the pulse until its echo is received, d is the distance from the transducer to the target and c is the speed of sound in the medium of interest. Rearranging (1) gives us $d = tc/2$ for the distance where the speed of sound in human tissues is 1540 m s^{-1} .

1.3.2.3 *Image formation*

Combining information of image position (i.e. which transducer element has received the echo) and echo range (i.e. the time since transmission) each echo is converted into a pixel of specific brightness which is then displayed on the screen. Converting all received echoes into pixels of defined brightness and mapping them on a two dimensional screen gives rise to the B-mode image (Martin 2003).

1.4 Doppler

1.4.1 The Doppler effect

The Doppler effect is the change in the observed frequency of sound when the object emitting the sound is moving. When the object emitting a sound is stationary the observed frequency of sound (f_r) is the same as the emitted frequency (f_t). However if the sound source is moving toward the observer the experienced frequency is higher as the sound waves become more compressed and the opposite happens if the source is moving away. This change in frequency is called the Doppler shift (f_d) and it is proportional to the relative velocity of the source to the observer. When the ultrasound beam is reflected off moving blood there are two Doppler shifts, one when the transmitted ultrasound strikes moving blood vessels and a second when moving blood vessels emit the reflected ultrasound. The Doppler shift frequency (f_d) depends on the velocity of sound in the medium (c), the transmitted ultrasound frequency (f_r), the velocity of blood (v) as well as the angle of insonation θ . This relationship is expressed as $f_d = f_r - f_t = \frac{2 f_t v \cos \theta}{c}$ which allows us to estimate the velocity of blood flowing through a vessel (Hoskins et al. 2003).

1.4.2 Doppler spectral analysis

In order to obtain the Doppler shift frequency the emitted ultrasound can be continuous or pulsed. The advantage of PW Doppler systems is that they allow the operator to specify the depth from which the Doppler shift frequency is obtained. Current ultrasound systems allow simultaneous B-mode imaging and PW Doppler analysis so that the area of Doppler analysis can be targeted more effectively. The conflict between the ideal angle of insonation which is

at right angles for B-mode imaging and parallel for Doppler analysis is resolved by angle correction which allows the Doppler beam to be steered up to 20° from the vertical. Once the Doppler signal is received by the transducer it is analysed into its frequency components by a process known as Fourier transformation. Spectral analysis consists of displaying consecutive Doppler frequency spectra on a time axis. As the angle of insonation is known the frequency scale can be converted to blood velocity (Hoskins et al. 2003; Kane 2009).

1.4.3 Colour Doppler imaging

In contrast to spectral analysis, which provides information from a single sample volume, colour Doppler imaging combines elements of B-mode image formation with PW Doppler to construct a colour coded image of flow in an area of interest. When colour Doppler is used the transducer emits two sets of ultrasound pulses, one set is used to construct the B-mode image and another set of lower frequency that is used to obtain Doppler shift information from the area of interest. Instead of Fourier transformation used in spectral analysis, colour Doppler imaging employs the technique of “autocorrelation”. This technique calculates three characteristics of the Doppler signal:

- i) The mean frequency of the Doppler shift
- ii) The power of the Doppler signal, which is proportional to the amplitude of the
- iii) The variance of the Doppler signal

The colour Doppler image can display any of these three outputs giving rise to corresponding image modes (Hoskins et al. 2003).

1.4.3.1 Power Doppler

The advantage of power Doppler over colour Doppler display is increased sensitivity. Because power Doppler is based on the display of the amplitude of the Doppler signal rather than the frequency shift it is not as susceptible as colour Doppler to aliasing and angle dependency (Rubin 1999).

1.5 Three dimensional ultrasound

3D ultrasound refers to the acquisition of a series of parallel B-mode images by the sequential movement of the transducer or of the ultrasound beam. These images are then saved as a 3D

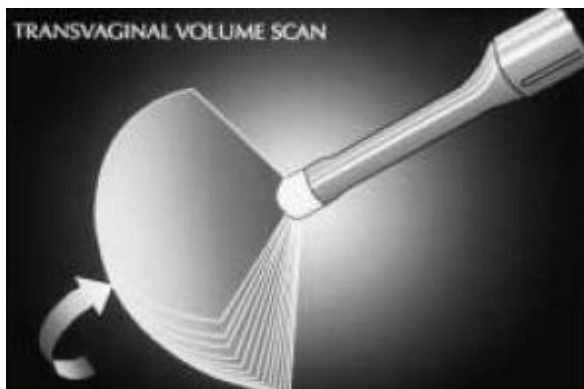


Figure 3 Schematic representation of 3D volume acquisition by transvaginal probe

volume of ultrasound information and can be manipulated using a computer processor. In the 1980s three dimensional scanning was restricted to a research setting but by 1989 the first three dimensional scanner became commercially available. This was called the Combison 330 and was launched by Kretztechnik AG (Feichtinger 1993; Maymon et al. 2000). Initially the three dimensional

ultrasound volume was obtained by an 180° rotation of the two dimensional scan plane around the long axis of the probe (Merz 1999). Although this provided accurate three dimensional images it was limited by merging of all images at the centre. In order to avoid this limitation current 3D probes include a mechanism to deflect the ultrasound beam and avoid merging. Once the 3D volume is acquired it is displayed in three orthogonal planes one

representing the original B-mode image and an additional 2 reconstructed planes at right angles to each other. It is possible to then rotate the image around a fixed point of interest as well as to move any of the three planes to obtain views that would not otherwise be possible because of the anatomical constraints of the female pelvis (Baba et al. 1997).

1.5.1 Three – dimensional ultrasound volume measurement

Another advantage of three- dimensional ultrasound over conventional two- dimensional ultrasound is the ability to measure the volume of irregular objects such as the endometrial cavity. This can be performed by a variety of methods depending on the equipment available. Using the Combison – 530 (Kretztechnik, Austria) the volume of the object of interest is manually delineated in a series of parallel 1 - 2 mm longitudinal sections. Once this is completed the volume is calculated by the built in computer. The validity and reliability of this method has been previously demonstrated by Gruboeck et al. (Gruboeck et al. 1996). A newer method for volume calculation has become available in the Voluson 730 which is the principal machine used in this thesis. It is called VOCAL and also requires the operator to delineate the object of interest. The difference here is that this is not done in a series of parallel slices but rather in a rotational fashion where the operator can define the rotation step from the options of 30°, 15°, 9° and 6°. Comparing the two methods in vitro for the measurement of the volume of objects of increasing complexity it has been shown that the rotational method demonstrates a trend toward increasing validity if a rotation step of 15° is used. Both methods tended to overestimate the volume of the object under examination in a systematic fashion depending on the irregularity of the object examined (Raine-Fenning et al. 2003). The in vivo reliability of the rotational method has also been assessed in a number of studies. VOCAL has proved to be superior to 2D technique of three distance measurement for

uterine volume estimation (Yaman et al. 2003). Bordes et al. in a series of 79 patients undergoing controlled ovarian stimulation showed that the intraobserver ICC for measuring the endometrial volume using the longitudinal plane and a rotation step of 15° was above 0.95 and better than using a rotation step of 30° (Bordes et al. 2002). Comparing the multiplanar method and the rotational method by repeated measures in a single premenopausal women, Raine – Fenning et al. confirmed high degrees of reliability and reproducibility for both techniques but showed that the rotational method with a 9° or 15° step was associated with less variance (Raine-Fenning et al. 2002). Raine – Fenning also showed that to achieve a balance between time taken to complete a study and accuracy a rotation step of 15° or 9° represents the best compromise. It could be argued that the endometrium in women undergoing controlled ovarian stimulation is a regular shape and therefore may be easier to delineate while in pathologic endometria this process will be more difficult because of the irregularity of the tumour and ill defined borders. Merce et al. compared the reproducibility of measuring endometrial volume with VOCAL between women undergoing controlled ovarian stimulation and those with potential endometrial pathology and found excellent ICC for intraobserver variability for both measurements (Merce et al. 2006). The specific technique, rotational vs. multiplanar, used to measure the volume of the endometrium does not seem significantly affect measurement reliability but does impact the time consumed to perform measurements. Further evidence for this comes from Cheong et al. who compared the interobserver and intraobserver variability for the measurement of fetal volume by VOCAL and the older multiplanar technique. They found that the two can be used interchangeably (Cheong et al. 2009). Clearly it is possible to achieve very high reproducibility for the analysis of digitally stored volumes. However to be clinically relevant a technique must not only be reproducible in terms of off - line analysis but

also in terms of data acquisition. This issue has been addressed by Raine – Fenning et al. (Raine-Fenning et al. 2004) and Martins et al (Martins et al. 2011) who found that there was no significant difference between observers in studies during which the transvaginal probe was removed and replaced between examinations by different operators. The latter group also showed that measuring the endometrial volume in either coronal or longitudinal plane did not produce significant differences. The authors suggested using the longitudinal plane as standard because this produces the most reproducible measurements with a relatively fixed posterior enhancement that is easier to avoid thereby preventing overestimation. Both in vitro and in vivo studies have assessed the validity and reliability of 3D ultrasound and VOCAL. The technique appears to be valid and also demonstrates good inter - and intraobserver agreement. Standardisation of technique has also been attempted. Despite this clinical applications for this technique have not yet been established.

1.5.2 Three dimensional power Doppler angiography

The Voluson 730 also allows the combination three – dimensional volume acquisition and concomitant power Doppler imaging. The only difference from the acquisition of a normal 3D ultrasound volume is that in addition to the B – mode ultrasound information the machine records the colour coded power Doppler signal. Post acquisition analysis can be either qualitative to identify the density of blood vessels as well as branching patterns (Sladkevicius et al. 2007) or quantitative. Current quantitative indicators are (Pairleitner et al. 1999):

$$\text{vascularisation index (VI)} = \frac{\text{colour voxels}}{(\text{total voxels} - \text{background voxels})}$$

$$\text{flow index (FI)} = \frac{\text{weighted colour voxels}}{\text{colour voxels}}$$

$$\text{vascularisation – flow index (VFI)} = \frac{\text{weighted colour voxels}}{(\text{total voxels} - \text{background voxels})}$$

According to the original description of these indices by Pairleitner et al. VI represents the number vessels in the tissue under examination, FI is a mean colour value that can characterise high flow intensity whilst VFI is designed to distinguish between instances of low vascularisation low flow and instances high vascularisation high flow. The relationship between these three indices and flow rate, medium concentration, distance between the region of interest and the transducer as well as the number of blood vessels in the region of interest has been studied in a flow phantom experiment by Raine – Fenning et al. (Raine-Fenning et al. 2008). They demonstrated that VI and VFI have a linear relationship with the concentration of scatterers in the medium under examination whereas FI varies in a curvilinear fashion. In clinical practice the scatterers are red blood cells and therefore variations in haematocrit between individuals or between examinations may reduce the validity of the indices. The same group demonstrated that in vitro increasing the distance between the transducer and the region of interest results in significant signal attenuation over a range of 20 – 80mm. The 3D – PDA indices demonstrated a significant relationship with the flow rate through the “blood vessels” as well as the number of “blood vessels” in the test tank. Apart from extrinsic factors the same group demonstrated that intrinsic factors such as machine settings (gain, power, PFR, WMF, signal rise, persistence, speed of acquisition) also affect 3D – PDA indices in a predictable manner. They conclude that in order to facilitate inter and intra subject comparisons of vascularity indices, machine settings should be standardised. It can be argued however that machine settings such as gain can be used to compensate for intra subject differences, such as BMI, that may result in variation. In practice such an approach would be difficult to standardise since as Martins et al have shown that

even in flow free experiments adjusting machine settings such as gain will return significant artefact (Martins et al. 2010).

1.5.3 Three dimensional power Doppler angiography in clinical practice

3D – PDA promises to allow objective quantification of organ vascularity which may have applications both for assisted reproduction and to enhance the diagnosis of pathological processes such as endometrial cancer. The histogram function of VOCAL has been used extensively in a variety of obstetric and gynaecological applications. Before evaluation of the diagnostic value of this technique in a clinical setting could be carried out, its reliability and reproducibility needed to be established. Raine – Fenning et al. in a study of 40 patients demonstrated good intraobserver and interobserver variability for the off – line analysis of 3D – PDA volumes acquired by a single operator with standardized settings (Raine-Fenning et al. 2003). Similarly to the issues surrounding 3D volume measurement, while it is possible to demonstrate high reproducibility for a stable digital dataset a major source of intraobserver variability will be the acquisition phase of the examination. To address this question the same group performed endometrial and ovarian volume acquisitions by 2 different operators on a series of 40 patients and demonstrated good interobserver reliability for the acquisition of 3D – PDA volumes (Raine-Fenning et al. 2004). Similarly Merce et al. (Merce et al. 2006) studied the intraobserver variability for the measurement of endometrial volume and 3D – PDA in 25 patients who were undergoing controlled ovarian stimulation and 15 patients with abnormal vaginal bleeding (either premenopausal or postmenopausal). They compared both a rotation state of 9° or 15° and different rotation patterns (antero – posterior vs. longitudinal axis) in the pre – acquired digitally stored datasets. They demonstrated that for all vascularisation indices (VI, FI, VFI) the intraobserver reliability is good with ICCs ranging

from 0.84 to 0.91. In fact they showed that the reliability of power Doppler indices measurement is significantly better for women with endometrial pathology compared to women undergoing controlled ovarian stimulation. Based on these results they concluded that the intraobserver variability for vascularisation indices is low and thereby reliability is high. More recently Opolskiene et al. studied 62 women with postmenopausal bleeding and endometrial thickness higher than 4.5mm. In these patients they acquired 3D – PDA volumes and analyzed them off line using VOCAL to determine the vascular indices in women with benign and malignant endometrial pathology (Opolskiene et al. 2010). They confirmed ICCs above 0.95 for all ultrasound variables. However a common denominator for all these studies is that intraobserver variability refers to the off line analysis of previously acquired and digitally stored volumes. While this is reassuring regarding the reliability of 3D – PDA for the off – line analysis of stored data it does not address the question of variation during the acquisition of the ultrasound information. As discussed above it has been shown in vitro that both machine settings (Raine-Fenning et al. 2008) and examination parameters such as distance between the transducer and blood vessels under examination (Raine-Fenning et al. 2008) will affect the magnitude of all indices. So, interobserver variability for the acquisition of the 3D – PDA volume rather than the analysis of already acquired volume may be higher than expected. For any study undertaken to examine the clinical value of 3D – PDA vascularity indices care must be taken to standardise the machine settings and examination technique to minimize variability in both acquisition and analysis of 3D – PDA ultrasound volumes. Lai et al in a study of the reproducibility of placental vascularity measurement using 3D – PDA (Lai et al. 2010) reported very poor reproducibility both for acquisition and analysis of already stored data. However some of the issues surrounding that study probably do not apply to the analysis of endometrial volume. Specifically, it is usually possible to

include all of the endometrium within one dataset which would preclude sampling error. This is not the case in imaging the placenta where the volume is substantially higher and choosing different areas to examine may result in different measurements. In an effort to achieve optimum acquisition Lai et al. varied the power Doppler gain between patients. The trade off of this study design is that unless the variation of gain is performed in a pre – agreed fashion it will have affected the reported 3D – PDA indices between examiners and therefore may be an additional source of poor reproducibility. In contrast to Lai et al, Raine – Fenning et al. demonstrated that for the acquisition of endometrial and ovarian 3D – PDA information the interobserver variability is low when machine settings are kept constant between patients. In conclusion, the findings in literature to date are reassuring regarding the reliability of 3D – PDA acquisition and analysis yet routine clinical applications for this modality are lacking.

[2] Uterine fibroids

2.1 Uterine fibroids

Fibroids are some of the commonest benign tumours, predominately found in the muscle layer of the uterus. As long ago as 1955 Miller et al. established that the origin of fibroids are the uterine smooth muscle cells (Miller et al. 1955). The nomenclature of fibroids reflects their cellular origin with various terminologies in use including leiomyoma and myoma. In histopathological terms fibroids are well circumscribed, pseudoencapsulated, solid masses that can reach up to 30cm in size and contain a large amount of connective tissue (Blake 2007). However, despite their widespread prevalence, fibroids' pathophysiology and growth determinants remain poorly understood.

2.1.1 Risk factors for fibroid development

A number of risk factors for the development of fibroids have been identified but the mechanistic link between risk factor and fibroid development remains speculative. It has been observed that the earlier the age of menarche the higher a woman's risk is to develop fibroids. This is thought to be due the increased number of menstrual cycles that promote fibroid growth through a higher number of cell divisions (Marshall et al. 1998; Wise et al. 2004). Parity is also inversely related to the risk of developing fibroids and this is assumed to be because of shorter periods of exposure to unopposed oestrogens in parous women (Flake et al. 2003). Older women tend to have more fibroids and at all ages the prevalence of fibroids

is up to 2 – 3 times higher in women of African origin (Baird et al. 2003). The increase in fibroid prevalence after 40 may be a consequence of accelerated fibroid growth in later years or be due to more women in that age group becoming symptomatic after 20 – 30 years of fibroid growth (Flake et al. 2003). Obesity is another risk factor for fibroids with most studies finding an increased risk for fibroids with increasing BMI (Parazzini et al. 1996; Wise et al. 2005). Diet is thought to play a role in fibroid development with women that consume more red meat and ham having moderately higher risk for uterine fibroids compared to women that consume more green vegetables and fish (Chiaffarino et al. 1999). Smoking reduces the risk of fibroids in a dose dependent way, an effect attributed to the anti oestrogenic effect of cigarettes (Ross et al. 1986). The literature on the relationship between oral contraceptives and fibroids is inconsistent with some reporting a protective effect, especially with increasing progesterone content (Ross et al. 1986) whilst others found no association (Parazzini et al. 1996; Wise et al. 2004). Similarly the effect of hormone replacement on fibroids is controversial: transdermal preparations seem to promote fibroid growth whilst oral preparations do not (Sener et al. 1996; Polatti et al. 2000; Ang et al. 2001). Tamoxifen use is also associated with an increase in fibroid size (Flake et al. 2003).

2.1.2 Heritability

Evidence of genetic predisposition toward fibroid formation exists. In a Finnish study of twins the concordance of hospitalisation due to fibroids was higher in monozygotic twins compared to dizygotic twins. However these authors found that anthropometric and environmental contributions were at least as important as genetic predisposition for the development of fibroids (Luoto et al. 2000). Other studies have found higher incidence of

fibroids in women with a first degree relative with fibroids (Vikhlyaeva et al. 1995; Sato et al. 2002).

2.2 Fibroid initiation

In the late 1960s and early 1970s X – linked glucose – 6 – phosphate dehydrogenase isoenzyme analyses revealed that fibroids had consistently one of the two X – chromosomes inactivated as opposed to random inactivation of either X – chromosome. This suggested that these tumours are monoclonal i.e. descended from a single myometrial cell. (Linder et al. 1965; Townsend et al. 1970). More recent studies, using X – chromosome methylation, show that in women with multiple tumours each nodule may arise from different original clones (Zhang et al. 2006; Cai et al. 2007) thereby confirming the monoclonal nature of each tumour. The specific trigger leading to fibroid development remains uncertain. Cytogenetic studies have revealed that a 40 – 50% of fibroids have a chromosomal aberration whilst the rest appear to have normal karyotype. It is suggested therefore that the initial events in fibroid development may be submicroscopic mutations although these have not been established yet (Rein et al. 1995).

2.2.1.1 Response to injury

Smooth muscle cells respond to injury by increased proliferation and production of extracellular matrix. The role of heparin binding growth factors, such as bFGF, and TGF – β is pivotal in this process (Stewart et al. 1998). The initiating injury can be secondary to a variety of factors including hypoxia or mechanical stress. Recent microarray analysis comparing normal myometrium to analogous fibroid gene expression revealed that around 25% of altered gene expression in fibroids is concerned with genes related to extracellular

matrix formation. In addition TGF – β expression was three times higher in fibroids compared to myometrium. This confirmed the results of previous studies that demonstrated a higher concentration of bFGF and TGF – β in fibroids (Mangrulkar et al. 1995). Another gene with altered expression in fibroids is the one encoding dermatopontin which is also underexpressed in keloid scars. The similarities between fibroids and keloids continue to a microscopic level as the extracellular matrix in fibroids demonstrates similar, disordered, morphological characteristics to the extracellular matrix in keloids. These observations led the authors to conclude that fibroids may be secondary to a disordered healing process leading to unchecked proliferation of myofibroblasts and the production of excess, disordered extracellular matrix (Leppert et al. 2006). It remains uncertain why not all women develop fibroids secondary to environmental insults that presumably are near universal. Given the heritability demonstrated in families and particular racial groups as well as the widespread nature of fibroids in the general population, it is likely that a degree of susceptibility to fibroids is genetically inherited and is then potentiated by environmental factors. It is possible that the monoclonal proliferation observed in fibroids occurs before the development of chromosomal rearrangements detectable by the light microscope (Mashal et al. 1994). We can speculate that the initial trigger for fibroid formation is a defective healing process driving cell proliferation that is then potentiated through cytogenetic changes.

2.2.2 Cytogenetic studies

The techniques used to examine the cytogenetic content of fibroids include classic genetic karyotyping which requires in vitro culture of fibroid cells and comparative genomic hybridisation. None of these techniques allows the identification of submicroscopic

chromosomal changes but nevertheless approximately 40 – 50% of fibroids examined have been found to have chromosomal abnormalities (Flake et al. 2003).

One of the commonest chromosome abnormalities in fibroids is the t(12;14) translocation which is also found in other benign solid tumours such as lipomas (Ligon et al. 2001). The disrupted region of chromosome 12 maps for the high mobility HMGIC gene (Ashar et al. 1995) which apart from fibroids is also expressed in rapidly proliferating adult human tissues such as lung and kidney (Gattas et al. 1999). However the precise function of HMGIC remains to be elucidated. The gene involved on chromosome 14 is the RAD51L1 (Schoenmakers et al. 1999) which is ubiquitously expressed in human tissues and is thought to play a role in regulation of the cell cycle (Rice et al. 1997). It is conceivable that a RAD51L1/HMGIC fusion given their purported roles in cell proliferation may result in dysfunctional cell growth (Flake et al. 2003).

Another common chromosomal abnormality in fibroids (around 17% in chromosomally abnormal tumours) is the deletion of chromosome 7 del(7)q22q32 (Flake et al. 2003). The area involved in the deletion is large and therefore it has been difficult to pinpoint specific genes involved in this deletion.

Other less common chromosomal changes found in fibroids include, among others, aberrations of 6p21 and trisomy 12. However 50 – 60% of fibroids show normal karyotype suggesting that either the genetic aberration is a secondary event to fibroid proliferation or that the primary events in fibroid pathogenesis are submicroscopic mutations (Xing et al. 1997; Ligon et al. 2001). The existence of genetic loci that confer susceptibility to fibroids is supported by heritability studies.

2.2.2.1 Correlation of fibroid genotype with phenotype

Some reports suggest that fibroids that demonstrate mitotic activity or have atypical histology are more likely to have karyotypic abnormalities (Pandis et al. 1990). Others have found an association between fibroid size and karyotype: Fibroids with normal karyotype are smaller than mosaic ones, which in turn are smaller than non mosaic fibroids. The same authors suggested an association between fibroid size and specific karyotype but these results failed to reach statistical significance (Rein et al. 1998).

2.3 Fibroid growth

The natural history of fibroids is not very well understood and a very small number of longitudinal studies on the subject exist (DeWaay et al. 2002; Peddada et al. 2008). Clinical observations as well as a survey of the risk factors for fibroid development point to a pivotal role for circulating ovarian steroids, oestrogen and progesterone.

2.3.1 Ovarian steroids

Traditionally oestrogen has been proposed as a promoter of fibroid growth. The risk of fibroids is greatest in women likely to have a high level of unopposed oestrogen such as nullips, those with early menarche or those who are obese. Furthermore GnRH analogues, which have a hypooestrogenic effect, tend to stop or reverse fibroid growth (Andreyko et al. 1988; Lethaby et al. 2001). Even though the levels of circulating ovarian steroid hormones in women with fibroids are not different from those in women without fibroids, the local oestradiol concentration in fibroids is elevated compared to homologous myometrium. This is perhaps due to lower levels of 17 β - hydroxy – steroid dehydrogenase which accelerates

conversion of oestradiol to oestrone. In normal myometrium this enzyme is up – regulated by progesterone in the luteal phase of the cycle something that does not happen in fibroids (Pollow et al. 1978; Otubu et al. 1982). Oestrogen also acts to increase the number of oestrogen and progesterone receptors in the myometrium. Accordingly the numbers of both receptors rise in the follicular phase whilst they fall in the luteal phase either because of lower oestradiol levels or because of a feedback effect of progesterone (Englund et al. 1998). The concentration of oestrogen and progesterone receptors is higher in fibroids compared to autologous myometrium while it is unclear whether receptors in fibroids are subject to the cyclical variation observed in normal myometrium (Englund et al. 1998). Even though fibroids have been found to have a higher proliferation index throughout the menstrual cycle there is no evidence that this mitogenic effect is directly mediated by oestrogen. It is known however that fibroids' proliferative index is higher in the secretory phase of the cycle (Kawaguchi et al. 1991; Nisolle et al. 1999). In addition, the regression effect produced by GnRH analogues can be abolished by concomitant medroxyprogesterone administration (Carr et al. 1993). The effect of progesterone can also be seen in pregnancy when a minority of fibroids increase in size and undergo “red degeneration” (Otubu et al. 1982; Strobelt et al. 1994). These findings have led some to suggest that the growth mechanism of fibroids includes a priming action for oestrogen, increasing the number of receptors available for a mitogenic effect mediated by progesterone (Rein et al. 1995; Flake et al. 2003).

2.3.1.1 Progesterone hypothesis

As discussed above fibroids demonstrate increased mitotic activity in the luteal phase of the cycle and a similar effect is observed in women treated with oral progesterone (Segaloff et al. 1949; Kawaguchi et al. 1985; Tiltman 1985; Kawaguchi et al. 1989). In contrast the anti-

progesterone drug RU486 induced fibroid shrinkage after 3 months of use (Murphy et al. 1993) while the administration of progesterone along with GnRH analogues reversed the shrinkage effect of the analogue (Friedman et al. 1988; Carr et al. 1993). Fibroids in women receiving progesterone containing HRT also demonstrate increased mitotic activity as opposed to fibroids in women receiving oestrogen only HRT (Lamminen et al. 1992). Fibroids have higher progesterone receptor content than autologous myometrium and the fibroid progesterone receptor content correlates with fibroid growth (Brandon et al. 1993; Ichimura et al. 1998).

The mechanism through which progesterone has its effect is unclear. It may be partly mediated by an increase in EGF as it has been demonstrated that fibroids have higher EGF content in the secretory phase of the cycle (Harrison-Woolrych et al. 1994). Furthermore, the product of the Bcl – 2 protooncogene is much more abundant in fibroids in the secretory phase than the proliferative phase of the cycle while it is absent from normal myometrium (Matsuo et al. 1997). Evidence now supports a critical role for progesterone along with oestrogen in the growth of fibroids (Rein 2000).

2.3.1.2 Cytokines and growth factors

Evidence exists that the effectors of ovarian steroids in fibroid growth includes locally produced cytokines and growth factors. Investigators have shown a higher concentration of TGF - β 3 in fibroids, which is thought to promote cell proliferation and extracellular matrix formation, compared to autologous myometrium. The concentration of TGF - β 3 increased in the secretory phase of the cycle which suggests it is modulated by progesterone (Arici et al. 2000).

EGF is found in similar levels in fibroids and myometrium but in contrast to myometrium, in fibroids the levels of EGF increase after exposure to progesterone (Harrison-Woolrych et al. 1994; Maruo et al. 2000). The level of EGF receptors in fibroids however display greater sensitivity to regulation by ovarian sex hormones than EGF receptors in myometrium as evidenced by the reduction of EGF receptors in fibroids of women treated with GnRH analogues. In fact the reduction of EGF receptors correlates with the shrinkage of fibroids suggesting that the ovarian sex hormone effect of GnRH analogues is partly mediated by EGF (Flake et al. 2003).

2.4 Clinical impact

The burden posed by fibroids on the population is understood when we consider that they are the leading indication for hysterectomy in the United States and in the United Kingdom (Vessey et al. 1992; Farquhar et al. 2002). Fibroids can cause a variety of symptoms and the main determinants of their symptomatology are position and size.

2.4.1 Abnormal vaginal bleeding

Some of the most significant symptoms of fibroids are menstrual disturbances, commonly heavy periods, that can lead to profound anaemia. It has been shown that the prevalence of submucous or intramural fibroids i.e. fibroids in or near the endometrial cavity, is much higher in women with such bleeding irregularities compared to women without any symptoms (Clevenger-Hoeft et al. 1999). Wegienka et al. (Wegienka et al. 2003) in a study of almost 1,000 women demonstrated that the presence of a fibroid increases the risk of menorrhagia and that this increase in risk is dependent on the fibroid's size. So a fibroid less than 2cm in diameter increases the risk of gushing type bleeding by 40% whilst the increase

in risk for a woman with a fibroid >5cm is 90%. These authors did not identify an increase in the risk of menorrhagia in women with submucous fibroids after adjusting for fibroid size. It appears that the presence of a fibroid is enough to cause menstrual disturbances even if it does not distort the endometrial cavity.

Several theories exist to explain bleeding disturbances in women with fibroids. Classically it has been suggested that the mechanism by which fibroids cause menorrhagia involves changes in the vascular architecture of the uterus including an increase in the venous plexus. It is now suggested that the bleeding disturbances caused by fibroids are mediated by the dysregulation of vasoactive substances produced by fibroids such as bFGF, VEGF and TGF- β (Stewart et al. 1996). Other theories to explain fibroid mediated menorrhagia include an increase in the endometrial surface area, interference with uterine contractility and endometrial ulceration over a submucous fibroid (Stovall 2001).

2.4.2 Pain and pressure

Once fibroids grow to a considerable size they can cause discomfort through pressure on adjacent organs such as the bladder, bowel, ureters and spinal nerves. During pregnancy 20 – 30% of fibroids under the influence of circulating steroid hormones show considerable rapid growth which may result in internal haemorrhage and intense pain (red degeneration). Finally, pedunculated fibroids may tort on their pedicle causing acute pain (Stovall 2001).

2.4.3 Reproductive function

The effect of fibroids on reproductive function remains controversial. Pritts et al. (Pritts 2001) undertook a comprehensive meta - analysis that demonstrated that the presence of a

submucous fibroid resulted in significantly lower pregnancy, implantation and delivery rates. In a subsequent systematic review 8 years later, the same authors (Pritts et al. 2009) showed that the presence of intramural fibroids also negatively impacts the chance of ongoing pregnancy in women undergoing IVF compared to sub - fertile women without fibroids. In contrast they confirmed that subserous fibroids have no impact on the rate of pregnancy. More recently Sunkara et al. in a systematic review including over 6000 IVF cycles found that the presence of an intramural fibroid reduces the chance of live birth by 20% (Sunkara et al. 2010). Nevertheless the evidence that removal of fibroids restores fertility is more limited. Retrospective data has shown that women who underwent removal of a submucous fibroid have fertility levels comparable to women without fibroids (Surrey et al. 2005) which in combination with the results of the systematic reviews supports removal of such fibroids. In contrast there is no evidence to support routine removal of intramural fibroids in women with sub – fertility.

2.4.4 Effect on pregnancy

The presence of fibroids doubles the risk of adverse pregnancy outcome such as first trimester bleeding, premature rupture of membranes, placenta abruptio, breech presentation, prolonged labour, low Apgar scores and low birth weight (Coronado et al. 2000).

2.5 *Diagnosis of fibroids*

Fibroids are commonly diagnosed clinically in the evaluation of women with menorrhagia or pelvic pain and pressure. However a number of differential diagnoses exist for these women including adenomyosis, leiomyosarcoma and ovarian tumours. Ultrasound is an effective procedure for the visualisation of the uterus and the detection of fibroids and allows the

distinction between fibroids and other pathologies which may present with similar symptomatology (McLucas 2008). In fact one of the earliest applications of ultrasound for medical imaging was in the diagnosis of pelvic masses (Donald et al. 1958).

2.5.1 Ultrasound

On B-mode ultrasound fibroids appear as discrete, globular structures with well defined

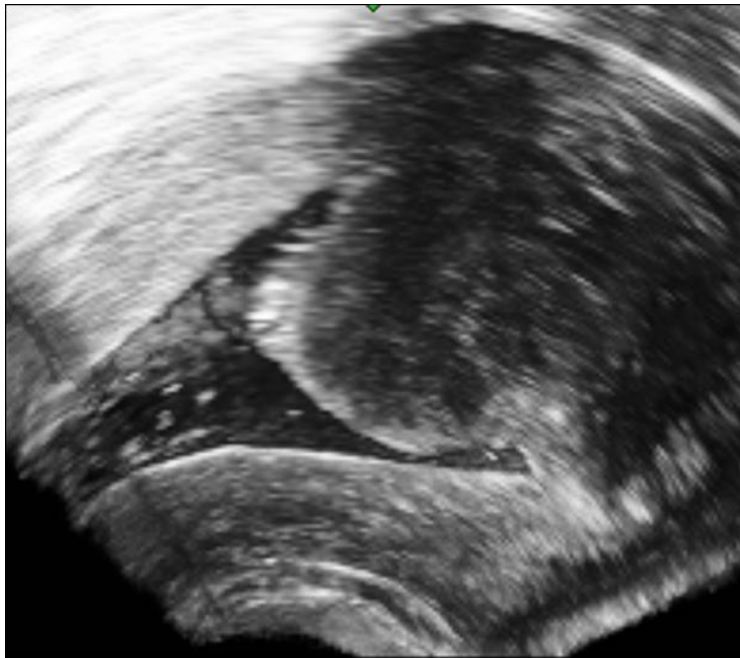


Figure 4 Transvaginal ultrasound image showing a submucous fibroid with saline infusion to delineate the endometrial cavity.

circumference (Figure 4). However they can have a variety of appearances depending on the ratio of connective tissue to smooth muscle and the presence and type of degeneration. Bizarre appearances can occur after fibroid degeneration when they can contain hypoechoic areas or calcifications. High resolution transvaginal ultrasound can identify fibroids as small as 4 – 5mm which are commonly

asymptomatic (Hurley 1998). The high diagnostic value of 2D transvaginal ultrasound was confirmed by Dueholm et al. in a study of 106 women scheduled for hysterectomy. The positive predictive value of ultrasound was 96% while ultrasound was accurate to within 2 mm of the true fibroid diameter as measured at histopathology (Dueholm et al. 2002). It

should be noted that the performance of ultrasound deteriorated when the uterine volume was large secondary to the presence of several tumours. This may be due to increasing distance between the transvaginal probe and the fibroid under examination. Despite its high sensitivity 2D grey scale ultrasound performs poorly in distinguishing between intramural and submucous fibroids as it is not always easy to discern the relationship between fibroid and endometrial cavity. Fukuda et al. showed that 2D transvaginal ultrasound misclassified 36% of fibroids when compared to hysteroscopic findings. Enhancing the image with the infusion of saline improved performance of 2D ultrasound and misclassification dropped to 2.8% (Fukuda et al. 1993). The enhancing effect of saline infusion for the diagnostic ability of 2D ultrasound was confirmed by Cicinelli et al who compared ultrasonic findings to those at the time of surgery (Cicinelli et al. 1995). They reported 100% sensitivity and specificity for sonohysterography and less than 2 mm difference between sonohysterography and direct evaluation after surgery. Other groups have reported similar findings (Dueholm et al. 2001). The high sensitivity of transvaginal ultrasound poses new problems for practitioners. Fibroids are often an incidental finding as tumours 5 mm in diameter do not cause symptoms. However they may become symptomatic if they grow to over 20 mm diameter (Wegienka et al. 2003). Given the dearth of information regarding the clinical course of these tumours with only two longitudinal studies of fibroid natural history published it is currently difficult for clinicians presenting a woman with an incidental diagnosis of a uterine fibroid to provide accurate information regarding the likely clinical course of the disease (DeWaay et al. 2002; Peddada et al. 2008). Further studies in the natural history of fibroids are needed to clarify these issues.

2.5.2 Other imaging modalities

MRI has been shown to be highly accurate for the diagnosis of uterine fibroids (Dueholm et al. 2002) and performs better than 2D ultrasound for the examination of the uterine cavity. However, MRI is equivalent to SIS and given the cost comparison with ultrasound it is not routinely used to achieve this diagnosis (Dueholm et al. 2001). In contrast, CT is not used for the diagnosis of fibroids as it has inferior diagnostic accuracy to ultrasound whilst involving ionizing radiation (McLucas 2008).

2.5.3 Fibroid classification

The position and size of a fibroid is of critical importance as it determines to a large extent the clinical impact of the tumour. Fibroids are classified into three broad categories based on their relationship with the endometrial cavity: intramural, subserous and submucous.

2.5.3.1 *Intramural fibroids*

Tumours that are confined to the myometrium are called intramural and are the commonest type. However as the myometrium is relatively thin (30 – 40 mm) growth of these tumours towards the serosal surface of the uterus or the endometrium changes their classification type (McLucas 2008).

2.5.3.2 *Subserosal fibroids*

Fibroids that protrude through the serosal surface of the uterus are called subserous. An extreme form of a subserous fibroid is a tumour connected to the uterus by a vascular stalk, called pedunculated fibroid. Subserous fibroids can acquire blood supply from other pelvic

organs, such as the bladder, in which case they are called parasitic. Large subserous fibroids can cause pressure while pedunculated fibroids may tort resulting in acute abdominal pain.

2.5.3.3 *Submucous fibroids*

Submucous fibroids are those that distort the endometrial cavity. They are associated with significant menometrorrhagia and are a cause of subfertility. Their classification was devised by Wamsteker et al. and it is based on the degree of protrusion of the fibroid into the endometrial cavity (Table 1). They used diagnostic hysteroscopy to subjectively evaluate the angle formed between the fibroid and the uterine wall for 51 submucous fibroids and deduced from this the proportion of fibroid protrusion into the endometrial cavity (Wamsteker et al. 1993).

Table 1 Current classification of submucous fibroids based on hysteroscopic findings

Type 0	Entirely in endometrial cavity
Type I	>50% in the endometrial cavity
Type II	≤50% in the endometrial cavity

Based on their results the authors claim that the higher the degree of protrusion of the fibroid into the endometrial cavity the fewer operations are necessary to achieve complete resection and once this is achieved it is likely to result in resolution of women's menorrhagia. Women with a type O fibroid had a 92% chance of complete resection per procedure compared to 60% chance for women with a type I fibroid and 50% chance per procedure in women with a type II fibroid (Wamsteker et al. 1993). A significant drawback of this classification system is

that although it readily identifies submucous fibroids that can be easily resected (Type O) it cannot be used to differentiate which of the remaining type I and II fibroids will also be resected easily. The authors concentrated solely on the degree of protrusion of the fibroid because they felt that the actual size of the fibroid could not be reliably assessed during hysteroscopy. This has been confirmed by Cicinelli et al., who found that hysteroscopic estimates of fibroid size differed significantly from actual measurements (Cicinelli et al. 1995). However incorporating some information regarding submucous fibroid size and shape in the classification may allow better discrimination between resectable and non – resectable tumours. Another disadvantage of this classification is that it is based on an invasive procedure, diagnostic hysteroscopy, which is associated with patient discomfort and a moderate risk of complications (Julian 2002). In order to avoid the need for diagnostic hysteroscopy, attempts have been made to use 2D transvaginal ultrasonography to classify submucous fibroids. These have shown that whilst this modality can provide information regarding fibroid size and position it cannot accurately estimate the degree of protrusion of the fibroid into the endometrial cavity. Saline infusion sonohysterography has been used to improve the diagnostic performance of 2D transvaginal ultrasound. This involves the insertion of saline solution in the endometrial cavity before the ultrasound scan is performed. De Kroon et al. found that in the investigation of women with menorrhagia 2D – SIS can replace diagnostic hysteroscopy in over 80% of cases. These women required no further investigation and thus were spared the discomfort of a more invasive procedure (de Kroon et al. 2003). It should be noted however that these investigators did not incorporate a gold standard in their study and thus it is possible some pathology was missed. More specifically to submucous fibroids Salim et al. in 2005 used 3D - SIS to preoperatively classify women with submucous fibroids based on the European hysteroscopic society classification (Table

1). All women then underwent diagnostic hysteroscopy. This study demonstrated good agreement between 3D- SIS and subsequent hysteroscopic findings (Salim et al. 2005). A year later Lee et al. demonstrated that 3D – SIS is a reproducible technique (Lee et al. 2006). Therefore not only can 3D - SIS obviate the need for diagnostic hysteroscopy for the classification of submucous fibroids, it remains to date the only technique that has been tested for reproducibility. Of course whilst a diagnostic method that is accurate and reproducible is highly desirable, the most critical question is whether the classification adopted is clinically relevant. As discussed above a classification based entirely on the degree of fibroid protrusion does not accurately differentiate between resectable and non - resectable fibroids. It is possible that the inclusion of further variables such fibroid size, shape and intramural portion depth may improve the diagnostic value of the classification. 3D – SIS provides a wealth of such information as the uterus can be evaluated in its entirety in contrast to diagnostic hysteroscopy. It remains to be seen whether it is possible to use this novel technique to improve the pre - operative staging of submucous fibroids.

2.6 *Fibroid treatment*

The mainstay of fibroid treatment is surgical removal (Vessey et al. 1992). Depending on the position of the fibroid this can be achieved either by an abdominal operation, open or laparoscopic, or a hysteroscopic operation as in the case of submucous fibroids. Currently medical treatment is limited to GnRH analogues. Because of their side effects, they can only be used for temporary symptomatic relief or pre operatively to facilitate operative removal.

2.6.1 Gonadotrophin hormone releasing analogues

GnRH analogues are derivatives of the naturally occurring GnRH. They contain peptide substitutions that increase their potency 40 – 200 times that of the native type. Initial exposure to GnRH analogues results in gonadotrophin release that transiently stimulates ovarian steroidogenesis. After 5 - 6 days, continued exposure of the pituitary to GnRH analogues results in a drop in serum gonadotrophins and a reduction of circulating oestradiol to postmenopausal levels (Shaw 1998). A recent meta - analysis of randomized controlled trials comparing either placebo or no treatment to pre operative GnRH analogues in women scheduled to have an operation for their fibroids (open myomectomy, open or vaginal hysterectomy) demonstrated significant benefits of pre - treatment with GnRH analogues including improved preoperative haemoglobin, reduced operative time for hysterectomy (but not myomectomy), reduced intraoperative blood loss during hysterectomy (but not myomectomy) and less vertical incisions for both types of operations (Lethaby et al. 2001). However pre-treatment with GnRH analogues is associated with significant side effects as a result of inducing a hypoestrogenic state in women including hot flushes, sweating, emotional lability and a reversible loss in bone mineral density (Matta et al. 1988; Shaw 1998; Sagsveen et al. 2003). It is not appropriate therefore to use GnRH in all cases of women with fibroids undergoing surgery but rather use should be limited to women with greatly enlarged uteri or with preoperative anaemia (Lethaby et al. 2000).

2.6.2 Selective progesterone modulators

As discussed above progesterone appears to play a critical role in fibroid growth. Based on this observation, selective progesterone modulators have recently been evaluated in clinical

trials as treatment for fibroids. Donnez et al compared the efficacy of ulipristal acetate to treat symptomatic fibroids with placebo (Donnez et al. 2012) and with leuprolide acetate (Donnez et al. 2012). Compared to placebo ulipristal acetate performed much better in controlling menorrhagia (92% vs. 19%) however it did not perform as well as leuprolide acetate (90% vs. 98%). As would be expected the side effect profile was preferable compared to leuprolide acetate with less menopause type symptoms. In addition to controlling symptoms ulipristal resulted in reduction of fibroid volume. However administration of ulipristal acetate was associated with histological changes in the endometrium while the effect on fibroid volume was temporary. The study was limited to 13 weeks so while ulipristal acetate may be an effective alternative to GnRH analogues for preoperative treatment it is unlikely to prove an effective long term treatment.

2.6.3 Transcervical resection of fibroid

Since the late seventies it is possible to remove submucous fibroids hysteroscopically thereby avoiding the morbidity associated with abdominal incisions (Neuwirth 1978). Long term follow up for this procedure is now available and it would appear that more than three quarters of patients treated for abnormal uterine bleeding require no further treatment after 5 years of follow up. It appears that the preoperative uterine size and the number of submucous fibroids are good predictors of the risk of recurrence as is completeness of resection. In contrast fibroid intramural depth does not predict the need for further surgery provided that the fibroid is completely removed (Emanuel et al. 1999). In a long term follow up study of over 200 women that underwent TCRF, Emanuel et al. found that 90.3% of women with a normal uterine size and not more than two submucous fibroids remained surgery free at five years compared to 64.8% of women with enlarged uteri and three or more fibroids. It appears

that in appropriately selected patients TCRF is an effective treatment for fibroid related bleeding disorders. However TCRF is associated with significant complications including the danger of excessive fluid intravasation that may result in fluid overload, pulmonary and cerebral oedema and even death. To minimize this risk strict fluid monitoring is performed intraoperatively. Some authors advocate preoperative treatment with GnRH analogues. Donnez et al. in 1989 published a longitudinal study of women scheduled for transcervical resection of fibroid that were treated with a GnRH analogue preoperatively. They concluded that pre-treatment reduces the submucous fibroid's surface area facilitating subsequent resection (Donnez et al. 1989). Although other authors (Andreyko 1988, Friedman 1989) have confirmed that treatment with GnRH analogues reduces fibroid size, it is not possible to conclude from the study by Donnez et al. whether pre-treatment reduces complications during TCRF as their sample size is very small (5 fibroids) and there is no control group. In contrast to these findings in a retrospective study of 283 first time transcervical resection procedures Emanuel et al. found that only operating time and proportion of intramural extension were significantly associated with the risk of fluid intravasation during the operation. They found no evidence that treatment with GnRH analogues preoperatively reduces fluid loss. Selection bias may have influenced the outcome of this study as it was observational and patients were not randomised to treatment with GnRH or placebo. The authors were unable to reliably estimate fibroid size hysteroscopically but suggested that this may be another variable that influences intraoperative fluid loss (Emanuel et al. 1997). The role of pre-treatment with GnRH analogues in women scheduled for TCRF remains uncertain. An adequately powered multicentre randomized controlled trial would be best suited to answer this question.

[3] Endometrial cancer

3.1 Endometrial cancer

Endometrial cancer poses a significant burden on the population as it is the commonest gynaecological cancer and the 4th commonest cancer in women (Landis et al. 1999). Most women with endometrial cancer are between 50 and 60 years of age and it is expected that with an ageing population the importance of this cancer will increase even further (Horn et al. 2007; Sorosky 2008). In 1983 Bokhman et al. suggested that the development of endometrial cancer follows two distinct pathways based on their observation of the characteristics of patients with endometrial cancer (Bokhman 1983). They found that obese women with signs of hyperoestrogenism such as anovulatory bleeding, infertility and late onset of menopause were more likely to develop highly and moderately differentiated tumours whilst women with none of those signs had a higher frequency of poorly differentiated and more aggressive type of cancer. Patients' characteristics as described by Bokhman are listed in Table 2. Although simplistic, this model is still in use today: Endometrial cancers are most frequently Type I, commonly endometrioid type and are oestrogen related occurring on a background of endometrial hyperplasia. In contrast, Type II cancers are rarer, consisting of high grade disease usually serous or clear cell histology, and occur in older women with no signs of hyperoestrogenism.

Table 2 Different characteristics of women diagnosed with the 2 principal types of endometrial carcinoma as described by Bokhman

Sign	Type I	Type II
Menstrual function	History of anovulatory bleeding	No disturbances
Reproductive function	Decreased, frequent infertility	No disturbances
Onset of menopause	Often after age 50	Often under age 50
Type of colpocytologic reaction in the postmenopause	Oestrogenic	Atrophic or transitional
Ovarian status	Hyperplasia of theca tissue Stein – Leventhal syndrome Feminising tumours	Fibrosis
Endometrial background	Hyperplasia	Atrophy
State of myometrium	Fibroid, internal endometriosis	No changes
Hyperlipidaemia	Yes	No
Diabetes mellitus	Yes	No
Hypertension	Associated with obesity/DM	No

3.1.1 Risk factors for endometrial cancer

It follows that factors that increase a woman's exposure to unopposed oestrogen increase the risk of developing Type I endometrial cancer. Early menarche and late menopause as well as nulliparity are all associated with an increase in the risk of endometrial cancer (Purdie 2006). Use of unopposed oestrogen as hormone replacement therapy has also been shown to increase the background risk. Grady et al. in a meta - analysis found that use of unopposed oestrogen replacement therapy gives a summary relative risk of 2.3 for the development of endometrial cancer and that this risk is proportional to the duration of oestrogen use (Grady et al. 1995). In contrast women who used progestins alongside oestrogen had a summary relative risk of 0.8. Increased BMI (>30) is associated with an increase in the risk of developing endometrial cancer with the relative risk in the range of 2 to 10. The effect of high BMI appears to be stronger in postmenopausal women (Kaaks et al. 2002). Diets with a high content in fat also increase the risk of endometrial cancer independent of their effect on BMI and the same applies to diabetes. In contrast, eating fruit and vegetables reduces that risk as does smoking (Purdie 2006). The protective effect of smoking is thought to be because of its antioestrogenic effect (Parazzini et al. 1995).

3.1.2 Precursor lesions

Endometrial hyperplasia is a non – physiological proliferation of endometrial glands. This may be simple or complex depending on the density of the glands. When there is atypia present the glands are highly irregular in size and shape with enlarged and rounded nuclei. Complex hyperplasia with atypia is considered to be a precursor for Type I endometrial cancer with a risk of progression between 28.6% and 46.2% (Horn et al. 2007). Endometrial

intraepithelial carcinoma is a non invasive glandular lesion characterised by epithelial cells with nuclear abnormalities similar to those seen in serous carcinoma. EIC differs from atypical hyperplasia in that it is found in older women with atrophic endometrium without any signs of hyperoestrogenism. EIC is considered to be a precursor lesion for Type II endometrial cancers (Ambros et al. 1995).

3.1.3 Molecular origin of endometrial cancer

Type I cancers form the majority (70 – 80%) of endometrial cancers (Liu 2007). They are usually low grade and have histological features reminiscent of the endometrium, hence they are called endometrioid. The most frequently altered gene in endometrioid cancer is the PTEN, located on chromosome 10 and coding for a protein with tyrosine kinase function. The role of PTEN involves arresting cell cycle progression, controlling apoptosis and inhibition of cell spreading (Mutter 2001). Clearly, loss of these functions may result in abnormal cell growth. The importance of PTEN is highlighted by the fact that loss of PTEN function is found in 83% of endometrioid cancers and up to 55% of precancerous lesions. Other important genetic alterations in Type I cancers include microsatellite instability and constitutive activation of the K-ras protooncogene among others (Liu 2007).

The remaining 10 – 20% of endometrial cancers are Type II. They are unrelated to oestrogen and arise on a background of atrophic endometrium. Patients with type II cancers are usually 5 – 10 years older and the commonest histological types are serous or clear cell which, are non - endometrioid. In Type II cancers the commonest genetic alteration is p53 mutation (Tashiro et al. 1997) whilst PTEN, MI and K-ras mutations are rare.

3.1.4 Histological subtypes

3.1.4.1 *Endometrioid carcinomas*

These are the commonest endometrial cancers comprising up to 84% of the total (Creasman et al. 2006). So - called because of similarities in their histological appearance with proliferative endometrium by definition contain less than 10% of other histological types. Macroscopically they are similar to other endometrial cancers forming focal (polypoid) or diffuse tumours that protrude into and distend the endometrial cavity (Rollason et al. 2006). Endometrioid cancers have a favourable prognosis with a five year survival for all stages of 83% compared to 53% for serous and 62% for clear cell carcinomas (Creasman et al. 2006)

3.1.4.2 *Serous carcinomas*

In contrast to endometrioid cancers, uteri with serous carcinomas often appear small. However serous carcinomas are associated with frequent myometrial invasion and even very small tumours have a poor prognosis with up to 13% of tumours confined to the endometrium having para – aortic lymph node metastases (Rollason et al. 2006).

3.1.4.3 *Clear cell carcinomas*

Clear cell carcinomas have no distinctive gross features. Microscopically they are comprised of clear cells with a high mitotic index. They have a similar prognosis to serous carcinomas.

3.1.4.4 *Mixed and undifferentiated carcinomas*

When a minor component of the tumour exceeds 10% of the lesion the tumour is then called mixed. Except for serous, clear cell and undifferentiated components the impact of this on

disease progression in unclear (Rollason et al. 2006). Undifferentiated tumours do not show distinctive histological features and comprise of solid collections of small or large cells. Their prognosis is dependent on stage of disease (Rollason et al. 2006)

3.1.5 Prognostic factors for endometrial cancer

3.1.5.1 *Stage*

The various stages of endometrial cancer in clinical application when research for this thesis was conducted are summarised in Table 3, however the various stages have been recently updated (Table 4) (Pecorelli 2009). Surgical staging remains the most important predictive factor for survival with 91% of stage Ia patients surviving five years compared to 85% of stage Ic. The large majority (86%) of endometrioid carcinomas are early stage cancers (stage I and II) compared to 59% for serous and 67% for clear cell types. In stages III and IV, however, endometrioid cancers represent 70% of all cancers while serous and clear cell represent 14% (Creasman et al. 2006). The difference in survival rates for different histotypes may be partly explained by serous and clear cell carcinomas presenting at more advanced stages, however even at stage I, endometrioid cancers have a 90% five year survival rate compared to 85% for serous and clear cell cancers (Creasman et al. 2006).

Table 3 FIGO staging of endometrial cancer in use when this thesis was carried out

Stage Ia	Tumor limited to the endometrium
Stage Ib	<50% myometrial invasion
Stage Ic	>50 % myometrial invasion
Stage IIa	Tumor extending to cervix involving only glands
Stage IIb	Tumor involving cervical stroma
Stage IIIa	Tumour invades the serosa of the corpus uteri and/or adnexae and/or positive cytological findings
Stage IIIb	Vaginal metastases
Stage IIIc	Metastasis to pelvic and/or para – aortic lymph nodes
Stage IVa	Invasion of bladder and/or bowel mucosa
Stage IVb	Distant metastases including intra – abdominal and/or inguinal lymph nodes

Table 4 Revised FIGO staging of endometrial cancer

Stage Ia	<50% myometrial invasion
Stage Ib	≥50 % myometrial invasion
Stage II	Tumor involves cervical stroma, does not extend beyond the uterus
Stage IIIa	Tumor invades the serosa of the corpus uteri and/or adnexae
Stage IIIb	Vaginal and/or parametrial involvement
Stage IIIc	Metastasis to pelvic and/or para – aortic lymph nodes
Stage IVa	Invasion of bladder and/or bowel mucosa
Stage IVb	Distant metastases including intra – abdominal and/or inguinal lymph nodes

3.1.5.2 Myometrial invasion

This is an important prognostic factor for Stage I cancers. Invasion of the outer third of the myometrium doubles the risk of recurrence compared to invasion limited to the inner third (Morrow et al. 1991). Because of the irregular shape of the uterus the distance from the tumour to the serosal surface may be a more appropriate method to report than myometrial invasion: the closer the tumour is to the serosal surface the worse the prognosis (Kaku et al. 1994).

3.1.5.3 Grade

Grading refers to the extent to which the tumour retains recognisable glands compared to solid areas. It is mostly applicable to endometrioid cancers as serous, clear cell and undifferentiated tumours are by definition high grade. Grade 3 tumours have considerably worse prognosis compared to Grades 1 and 2. For example the risk of pelvic lymph node increases from 1.43% for a Grade 1 lesion without myometrial invasion to 37% with a Grade 3 lesion with deep invasion (Morrow et al. 1991) (Table 5).

Table 5 Histopathological grading of endometrial cancers

Grade 1	5% or less solid areas
Grade 2	6 – 50% solid areas
Grade 3	more than 50% solid areas

3.1.5.4 Cervical involvement

It is thought that involvement of the cervix in patients without extrauterine involvement increases the risk of recurrence. However it has not been shown that this effect is independent of tumour grade and myometrial invasion (Morrow et al. 1991).

3.1.5.5 Lymph node involvement

Involvement of pelvic and para – aortic nodes doubles the risk of recurrence (Kaku et al. 1994).

3.1.5.6 Tumour size

The contribution of tumour size to the stage of endometrial cancer has been recognised since the earliest attempts at staging this disease (Gusberg et al. 1960). Attempts at preoperative measurement of the size of endometrial cancer have been reported and have demonstrated a correlation between tumour size and disease prognosis (Johnsson et al. 1979). Although it does not form part of the formal staging process as outlined in Table 3, the size of tumour is known to influence the chance of lymph node involvement and thus cancer prognosis. Schink et al. in a study of 91 patients showed that the lymph node metastases occurred in 5.7% of patients with tumours less than 2 cm in diameter, in 21.7% of patients with tumours more than 2cm in diameter and 50.0% of patients with tumours filling the endometrial cavity. Using multivariate analysis they demonstrated this effect to be independent of the depth of myometrial invasion although larger tumours tended to have deeper myometrial infiltration (Schink et al. 1987).

3.2 *Diagnosis of endometrial cancer*

The typical presentation of endometrial cancer is post – menopausal bleeding and the reported incidence of endometrial cancer in women with this symptom is between 5% and 15% (Lidor et al. 1986; O'Connell et al. 1998). A variety of diagnostic procedures have been proposed to investigate these women and identify those with endometrial cancer.

3.2.1 Blind endometrial biopsy

Introduced by Recamier in 1843 D&C has been the mainstay diagnostic option for the investigation of women postmenopausal bleeding for decades. Once the most commonly performed gynaecological diagnostic procedure in the last three decades this practice has become obsolete for a number of reasons. Apart from being an invasive procedure that requires a general anaesthetic, it has been shown that D&C is inadequate for diagnosing intrauterine pathology with a sensitivity as low as 46% (Lerner 1984; Emanuel et al. 1997; Bettocchi et al. 2001). Not only does D&C have poor sensitivity but it is also associated with a significant risk of complications up to 1.7%, with uterine perforation occurring in almost 1% of cases. Moreover it is an expensive procedure involving day case hospitalisation which causes considerable disruption in patients' lives (Grimes 1982). For all these reasons D&C is being abandoned in favour of other procedures.

Another method to obtain an endometrial specimen which can be done in the outpatient setting, thereby avoiding a general anaesthetic, is to use a Pipelle. This is a transparent, flexible tube 23.5 cm long containing an inner piston. Withdrawing the piston once the device is in place in the endometrial cavity creates negative pressure which draws endometrial contents into the tube. The sensitivity of Pipelle biopsy has been reported to be quite high, up

to 97.5%, but these studies were performed in patients with known endometrial cancer and thereby may suffer from operator bias (Stovall et al. 1991; Guido et al. 1995). Despite this high sensitivity in the study by Guido et al. 11 patients with endometrial cancer would have been missed. 5 of those 11 had a cancer in localised in a polyp while 3 had disease in less than 5% of the endometrial cavity. Rodriguez et al. quantified the area sampled by Pipelle biopsy to a mean 4.2% which would explain why localised lesions can be missed (Rodriguez et al. 1993). Clearly Pipelle biopsy is a useful method to obtain an endometrial specimen however it should used only when the diffuse nature of the lesion has been confirmed by imaging (Lee et al. 2006).

3.2.2 Hysteroscopy

Hysteroscopy is an invasive procedure performed under anaesthesia that allows direct visualisation of the endometrial cavity and targeted sampling of suspicious areas. As an alternative the procedure can be performed in the outpatients with patient satisfaction that is equivalent to day case procedures but significantly shorter recovery (Kremer et al. 2000). Outpatient hysteroscopy remains however an invasive procedure associated with significant patient discomfort (Tahir et al. 1999). In one study of over 2,500 outpatient hysteroscopies of patients with abnormal vaginal bleeding, including post menopausal women, in almost 50% of patients the result was a normal endometrial cavity (Nagele et al. 1996). Clark et al. in a recent meta - analysis involving over 26,000 patients found that the sensitivity of hysteroscopy for the detection of endometrial cancer was 86.4% with a specificity of 99.2% giving a positive likelihood ratio of 60.9 and a negative likelihood ratio of 0.15 (Clark et al. 2002). However the majority of studies reported used endometrial biopsy obtained at the time of hysteroscopy as the gold standard. Lo et al. in a study involving 1600 patients scheduled

for outpatient hysteroscopy had a failure rate of almost 10% and found intrauterine pathology in only 17% of patients. Their positive predictive value for endometrial cancer was 21% (10/48) and of the 20 cases of endometrial cancer detected on endometrial sampling only 10 were correctly identified on hysteroscopy. Lo et al. recommended that endometrial biopsy be performed in every hysteroscopy as the positive predictive value of hysteroscopy is not adequate on its own. It is certainly difficult to see the advantage of performing a diagnostic hysteroscopy without obtaining an endometrial specimen. In view of the invasive nature of the procedure and the cost involved in hysteroscopy it would be preferable to use this procedure as a second line test in women at high risk of endometrial cancer.

3.2.3 Transvaginal ultrasonography

The development and availability of transvaginal ultrasound offered a non invasive procedure that can accurately image the uterus and endometrium. In contrast to diagnostic hysteroscopy, transvaginal ultrasound has no complications and is well tolerated by patients (Nasri et al. 1991). So, in view of the inadequacies of D&C and the fact that up to 83% of women undergoing an invasive procedure such as diagnostic hysteroscopy were not found to have endometrial pathology (Lo et al. 2000) transvaginal ultrasound has been evaluated as a possible first line assessment of women with postmenopausal bleeding (Emanuel et al. 1995). In 1990 Osmer et al. scanned 103 post menopausal women with post menopausal bleeding and established that in women with an endometrial thickness of over 4mm the risk of endometrial cancer increased significantly (Osmer et al. 1990). Similar results were reported by Nasri et al. a year later (Nasri et al. 1991). Around the same time Goldstein et al. reported that in postmenopausal women with bleeding, if the endometrium is <5mm on transvaginal scan minimal tissue is obtained on sampling (Goldstein et al. 1990). However, both Osmer

and Nasri used curettage as their gold standard which may have led them to miss a substantial proportion of cancers given the low diagnostic yield of this procedure (Grimes 1982). In order to remedy this deficiency Emanuel et al. performed a study of the predictive value of transvaginal ultrasound using outpatient hysteroscopy and biopsy for all patients as the gold standard. In this study all 6 women with postmenopausal bleeding who were diagnosed to have cancer on hysteroscopy had an abnormal transvaginal scan (Emanuel et al. 1995). However 16 women with an abnormal transvaginal scan had a normal diagnostic hysteroscopy. Nevertheless using transvaginal ultrasound as first line investigation of women with postmenopausal bleeding would have reduced the rate of an invasive procedure such as hysteroscopy by 50%. In 1995 the first of a series of multicentre trials to evaluate transvaginal scanning as the initial investigation of women with postmenopausal women was reported. In 1138 women with postmenopausal bleeding 114 endometrial cancers were diagnosed by curettage and none of these women had an endometrial thickness less than 5mm (Karlsson et al. 1995). Using a 4mm cut off the positive predictive value for detecting any endometrial pathology was 61% whilst the negative predictive value was 97%. Similar results were reported by Ferrazzi et al. a year later in a study designed to reflect actual clinical practice and a variety of sonographic expertise levels (Ferrazzi et al. 1996). In 2002 Gupta et al. published a meta-analysis that included 57 studies with 9031 patients. They found a pre-test probability of 14.0% for endometrial cancer in this population of women while the likelihood ratio for a negative test result was 0.15 (5mm cut-off). However the likelihood ratio for a positive test result using the same cut-off was 2.7 giving a probability of endometrial cancer in a woman with a positive test result of 26.1%. They concluded that whilst transvaginal ultrasound is a good test to rule out endometrial cancer it is not a good test to rule the disease in (Gupta et al. 2002).

3.2.4 Transvaginal ultrasound with Doppler

Bourne et al. compared the uterine artery PI in women with postmenopausal bleeding and found that PI is reduced in women with endometrial cancer. They concluded that transvaginal Doppler has great potential in the assessment of uterine pathology (Bourne et al. 1991). However it is unclear from their methods how they selected their patients. In subsequent studies neither Sladkevicius et al. nor Sheth et al. confirmed the value of Doppler examination for the differentiation of benign pathology and endometrial cancer (Sladkevicius et al. 1994; Sheth et al. 1995). Power Doppler, which does not suffer from aliasing and has better ability to delineate tortuous vessels, has been used to qualitatively differentiate the vascular pattern in women with malignant and benign endometrial lesions. Epstein et al. in 2002 used 2D power Doppler to develop a subjective and objective probability of malignancy in women with an endometrial thickness over 5 mm. They concluded that inclusion of a power Doppler variable can contribute to the correct diagnosis of endometrial malignancy (Epstein et al. 2002). A year later, Alcazar et al. reported that in more than 80% of cases of endometrial cancer the endometrium displayed a specific vascular pattern on power Doppler analysis (Alcazar et al. 2003). In 2006 Epstein et al. used colour Doppler to analyse the vascular pattern of endometrial tumours and did not find this was helpful to differentiate between benign and malignant endometrial tumours (Epstein et al. 2006). Of course this may reflect a weakness of colour Doppler compared to power Doppler.

3.2.5 Three – dimensional ultrasound

One of the earliest studies of the use of 3D ultrasound for the diagnosis of endometrial cancer was the study performed by Gruboeck et al. in 1996 (Gruboeck et al. 1996). They studied 107

women with postmenopausal bleeding and measured both endometrial thickness and endometrial volume. They found that volume measurements performed better than endometrial thickness, a cut – off of 13ml would diagnose all endometrial cancers with a single false positive giving a sensitivity of 100% and specificity of 98.8%. The best cut - off for endometrial thickness was 15 mm with a sensitivity of 83.3% and specificity of 88.2%. Despite these encouraging results the next study of 3D ultrasound for the diagnosis of endometrial cancer was performed over 10 years later by Mansour et al. in 2007. They studied 170 women with postmenopausal bleeding comparing endometrial thickness and endometrial volume for the prediction of endometrial cancer. They found that the sensitivity of endometrial thickness for the prediction of endometrial cancer was 79% compared to 91% for endometrial volume. However they used dilatation and curettage as their gold standard which has been shown to be unreliable (Grimes 1982) and also did not specify the cut – off value used for endometrial thickness nor provide the specificity of the test at that sensitivity (Mansour et al. 2007). Given the widespread use of 2D measurement of endometrial thickness and the acceptance of this test following a large meta – analysis (Gupta et al. 2002) it is unlikely that endometrial volume will be used to replace thickness as a primary test but it may be used as an adjunct in patients that test positive (Mansour et al. 2007). Other investigators have used the novel technique of 3D – PDA that allows quantification of endometrial perfusion as a second line test in high risk women. Odeh et al. included 145 peri - and post - menopausal women with an endometrial thickness >4mm and performed angiography to determine the VI, FI and VFI of the endometrium along with the endometrial volume (Odeh et al. 2007). They report a significant difference in endometrial volume, VI and FI in women with “normal” histology and endometrial cancer. Using a cut – off endometrial volume of 3.6 ml they report a sensitivity of 93.1% and specificity of 36.2%.

They conclude that 3D - PDA analysis is a good diagnostic tool in predicting endometrial carcinoma. However their results do not justify this statement as they included peri - menopausal women with post - menopausal women and their reported ROC AUC did not exceed 0.85 which necessary for a diagnostic test to be considered a good. Because they defined post - menopause as 12 months of amenorrhea after the age of 40 and included peri - menopausal women in their analysis the prevalence of endometrial cancer in their population was less than 10% (11 cases) which is low for a population of post - menopausal women with endometrial thickness >4mm. Finally they used a variety of diagnostic procedures and operators as their gold standard which introduces unreliability. Therefore this report is not useful in the assessment of 3D -PDA analysis. Alcazar et al. in 2009 used 3D – PDA as a second stage test in 99 women who presented with postmenopausal bleeding (Alcazar et al. 2009). 44.1% of their patients had a histological diagnosis of endometrial cancer and an additional 13.1% endometrial hyperplasia whilst the remaining patients had benign diagnoses (polyp, fibroid, cystic atrophy). The mean endometrial volume, VI and VFI were significantly higher in women with endometrial cancer compared to benign pathology and the AUC for prediction of endometrial cancer by VI alone was 0.902. These investigators concluded that VI has the potential to be a second line test in high risk women with postmenopausal bleeding. However the high rate of endometrial cancer in the population of this study suggests that the population studied is not representative of the general population but rather may be selected. Even though measurement of endometrial volume and application of 3D – PDA have shown promise as second line tests in women at high risk of endometrial cancer it is not possible yet to avoid endometrial biopsy based on one or a combination of these parameters in women found to be at high risk of endometrial cancer on 2D ultrasonography.

3.3 *Treatment of endometrial cancer*

3.3.1 Surgical treatment

The mainstay of treatment for endometrial cancer is surgical with total abdominal hysterectomy and bilateral oophorectomy. Current FIGO recommendations for the staging of endometrial cancer include pelvic and para - aortic lymph node biopsy in attempt to define the prognosis and the need for adjuvant therapy (Shepherd 1989). However performing a lymphadenectomy increases the operative time, the risk of intraoperative haemorrhage and the risk of lymphoedema (Ryan et al. 2003; Kitchener et al. 2009). Patients who had their lymph nodes removed have increased radiotherapy related complications (Creutzberg et al. 2000). In early cancers (stage I) the risk of lymph node involvement is variable from 0% in superficial grade 1 tumours to 25% in deep grade 3 tumours. Given the variation in the risk of nodal involvement, performing lymphadenectomy as a routine in all cases remains controversial. On this basis, some have suggested that the decision to perform lymphadenectomy in stage I tumours should be tailored to each individual's risk of nodal involvement (Creasman et al. 1987) as this would avoid lymphadenectomy in patients unlikely to have lymph node involvement thereby sparing these patients operative and postoperative complications. Given that the majority of endometrial cancers are early stage some form of patient selection for lymphadenectomy appears therefore desirable (Hacker et al. 2006). In a retrospective study of over 12,000 patients Chan et al. demonstrated a limited therapeutic effect of lymphadenectomy across all stages of endometrial cancer (Chan et al. 2006). However these authors could not control for standard of care, co-morbidity and stage migration. They also do not report the rate of postoperative complications in these patients. The therapeutic role of lymphadenectomy is therefore not currently widely accepted.

3.3.2 Adjuvant therapy

Most endometrial cancers are diagnosed at stage I. The definitive treatment of these patients after surgery remains unclear. Some may receive external beam radiotherapy or vaginal brachytherapy as an adjuvant to reduce the risk of recurrence and this decision is based on prognostic factors such as the stage of disease, the depth of invasion of the myometrium, grade of the tumour, age and lymphovascular invasion. However 26% of patients receiving adjuvant therapy report post - operative complications compared to only 4% of patients who did not receive it (Creutzberg et al. 2001). The risk of complications after adjuvant therapy increases further for patients who underwent lymphadenectomy (Greven et al. 1997). At the same time it remains unclear which patients benefit from such treatment. The PORTEC study included 715 women with stage I, grade 1 with deep invasion, grade 2 with any invasion and grade 3 with superficial invasion who did not undergo lymph node sampling. Patients were randomised to external beam radiotherapy or no further treatment. The rate of late complications was 25% in the radiotherapy group vs. 6% in the control group. Even though the rate of loco-regional relapse was higher in the control group (14% vs. 4%), the 5 year survival was similar in the two groups (85% vs. 81%). In view of these findings PORTEC concluded that radiotherapy can be omitted in younger patients (<60 years) with superficially invasive grade 2 tumours (Creutzberg et al. 2000). In a meta - analysis of four trials including 1770 patients with stages ranging from Ib/Ic to occult stage II and grade 1 to grade 3, there was no difference in overall survival between radiotherapy and control groups despite a reduction in loco-regional recurrence in patients who received adjuvant therapy (Kong et al. 2007). The most recent randomized controlled trial of external beam radiotherapy in patients with early cancers at intermediate (IA, IB grade 3 and IC IIA grade 1

and 2) or high risk of recurrence (IC and IIA grade 3, all women IIB) confirmed conclusively that external beam radiotherapy does not confer a survival benefit to these women and that the reduction in loco-regional recurrence does not justify the toxicity of external beam radiotherapy (Blake et al. 2009).

3.3.3 Preoperative staging

In view of the fact that surgical staging increases operative morbidity and that the stage of disease is of critical importance to decide whether adjuvant therapy is needed or not, attempts have been made to stage endometrial cancer pre operatively through imaging. A meta - analysis of 47 studies comparing MRI, CT and ultrasound for the staging of endometrial cancer showed no significant difference in the performance of these modalities except for the estimation of myometrial invasion in which contrast – enhanced MRI performed better than CT (Kinkel et al. 1999). However, in regards to ultrasound, this meta-analysis included studies using both transabdominal and transvaginal approaches and a variety of probe frequencies which will have affected the accuracy of reported results. More recently, the sensitivity of MRI in staging endometrial carcinoma was reported to be between 83 and 92% (Manfredi et al. 2005). In a recent study of 88 women with endometrial cancer stages I – IIB that compared 2D transvaginal ultrasound and MRI Savelli et al. showed that the two diagnostic modalities had equivalent performance for the prediction of myometrial invasion (84% sensitivity with 83% specificity for ultrasound compared to 84% sensitivity with 81% specificity for MRI). However transvaginal ultrasound performed better for the prediction of cervical involvement (93% sensitivity with 92% specificity for ultrasound compared to 79% sensitivity and 87% specificity for MRI) even though neither modality was able to distinguish between stage IIa and IIB (Savelli et al. 2008). A preoperative staging investigation of value

in the diagnosis of endometrial cancer would need to be able to distinguish between early (stage I – IIB) and late cancer in order to guide operative and postoperative management. Although this study demonstrates that in principle transvaginal ultrasound may be useful in the staging of endometrial cancer the fact that no patients with late cancers are included puts in question the clinical value of the findings. In recognition of this other investigators have constructed statistical models to differentiate between patients at stages Ia - Ib and Ic since this may have important consequences on the treatment pathway for these patients. De Smet et al. constructed a multivariate logistic regression model which retained the degree of differentiation, the endometrial volume, endometrial thickness and the number of fibroids as significant predictors. This achieved an area under the curve of 0.66 that improved to 0.77 with more complicated models (least squares support vector machines) (De Smet et al. 2006). These authors used 2D transvaginal ultrasound and estimated endometrial volume using the formula for an ellipsoid. However endometrial cancer tumours are likely to be irregular in shape and thus this method of estimation may be considerably inaccurate. Nevertheless this study showed the promise of measuring endometrial volume for preoperative staging of endometrial cancer.

PART II Materials and Methods

[1] Materials and methods

1.1 Setting

1.1.1 The Early Pregnancy & Acute Gynaecology Unit, King's College Hospital

The majority of work in this thesis was carried out at the EPAGU at King's College Hospital between August 2006 and February 2008. KCH is located in South London and serves the needs of the population of the boroughs of Lambeth, Southwark and Lewisham, around three quarters of a million. The local population is one of the most deprived in the United Kingdom with high levels of morbidity. The hospital was founded in 1840 and currently has 950 beds whilst it employs over six thousand staff with a budget of approximately £500 million. The EPAGU receives referrals for both early pregnancy and gynaecological complaints and over 10,000 women are seen annually.

1.1.2 The Gynaecological Diagnostic & Treatment Unit, University College Hospital

A part of this MD thesis was carried out at the GDTU at UCH. The GDTU forms part of University College Hospital which formed as a Trust in 1994. UCH is a central London teaching hospital with 595 inpatient beds that acts as a tertiary centre for referrals for the boroughs of Camden and Islington. The Trust has a turnover of £632 million, treats 500,000 inpatients a year and employs 6,000 staff.

1.2 Two dimensional transvaginal ultrasound

For this thesis three different ultrasound machines were used: The Combison 530 3D (Voluson, Kretztechnik, Austria), the Voluson 730 Expert (GE Medical Systems, Milwaukee, WI, USA) and the Voluson E8 (GE Medical Systems, Milwaukee, WI, USA). All transvaginal ultrasound examinations were performed in the lithotomy position by trained gynaecologists. Women who were virgo intacta were offered a transrectal examination. All examinations were performed with a 7.5 - MHz probe with 3D facility. First the uterus was examined in the transverse plane to identify the cervical canal and the uterine cavity. Acquired uterine anomalies, such as fibroids or adenomyosis, were diagnosed based on direct visualisation using previously described diagnostic criteria (Hirai et al. 1995). The probe was then rotated 90° anti – clockwise and the uterus and endometrium were visualised in the longitudinal plane. Care was taken to identify both layers of endometrium at the thickest point between the endometrial – myometrial junction. Once the thickest point was identified a measurement of both layers of the endometrium was taken. We did not include the surrounding low amplitude echo area (Lee et al.). If free fluid was present in the endometrial cavity, the fluid was measured separately and subtracted from the total thickness (Lee et al.). Pre – menopausal women with a suspected submucous fibroid were referred to a dedicated clinic for 3D – SIS. Post – menopausal women presenting with bleeding and an endometrial thickness $\geq 5\text{mm}$ were referred for 3D ultrasound and 3D - PDA.

1.3 Three – dimensional ultrasound

All 3D, 3D – PDA and 3D – SIS ultrasound volume acquisitions were performed by a single operator (DM) in automatic mode with maximum sector setting 120°. Similarly all off – line analyses were performed by the same single operator to minimise variability.

1.3.1 Three dimensional ultrasound

A conventional 2D transvaginal scan was performed using a 7.5MHz probe with 3D facility. First the midline longitudinal plane was identified and then the probe was held steady. Effort was made to include the entirety of the uterus and cervical canal in the volume acquired. Then the patient was asked to hold her breath and volume acquisition was switched on. The 3D volumes were generated by automatic rotation of the mechanical transducer through 180°. The acquired image was reviewed for quality and if satisfactory it was stored for later analysis. The acquired volumes were in the shape of a truncated cone with a depth of 4.3 – 8.6 cm and a vertical angle $\alpha=120^\circ$.

1.3.2 Three - dimensional power Doppler angiography ultrasound

The acquisition of 3D – PDA was similar to the acquisition of regular 3D volumes (Figure 5). In order to improve the reliability of the technique the minimum distance between the tip of the probe and the endometrium was sought by gentle pressure on the probe. Care was taken to include all of the endometrium including any areas of pathology within the ultrasound volume. Once the desired image was obtained the patient was asked to hold her breath to avoid motion artefact. Then the power Doppler gate was activated and positioned over the object of interest so that it was completely enveloped. The power Doppler settings were kept

constant between patients (power Doppler gain -0.2, pulse repetition frequency 0.6 kHz, WMF low). The acquisition was repeated if there was obvious motion artefact. When a good quality image was obtained it was stored for later analysis.

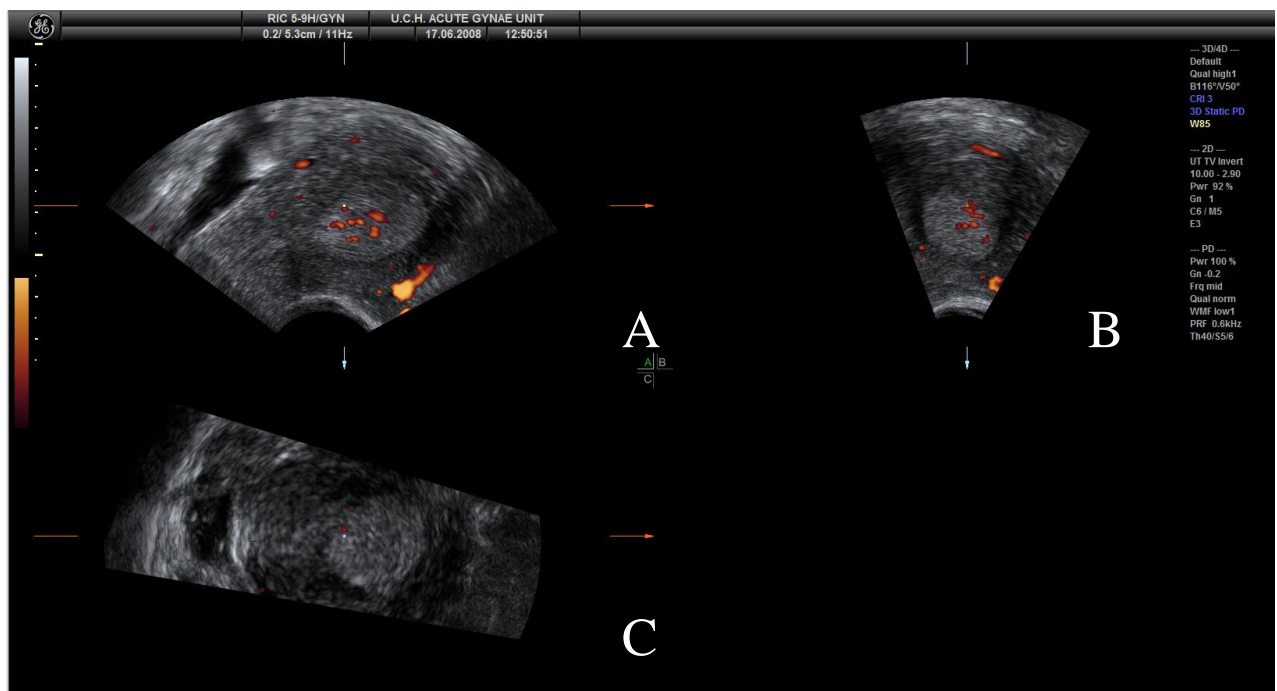


Figure 5 Image capture of 3D - PDA off line analysis demonstrating reference planes A (longitudinal), B (transverse) and C (coronal). For volume and 3D - PDA indices calculation we used reference plane A

1.3.3 Three – dimensional saline infusion sonohysterography

For women with a suspected submucous fibroid 3D – SIS was performed. First a sterile Cusco speculum was passed to visualize the cervix. Sterile chlorhexidine solution was used

to clean and then a 3.3mm soft plastic paediatric naso -gastric suction catheter was passed through the cervix into the uterine cavity without grasping the cervix. The speculum was removed and the 7.5MHz transvaginal 3D-ultrasound probe inserted into the vagina. The uterine cavity was visualized and the position of the catheter within the uterine cavity confirmed. A longitudinal view of the uterus was obtained and the catheter was withdrawn to a level just above the internal cervical os. A volume of 5–10 ml of sterile saline solution was then instilled into the uterine cavity. A 3D volume was generated by the automatic sweep of the mechanical transducer. The volume was saved for later analysis.

1.3.4 Off - line volume analysis using 4D view for endometrial volume and 3D – PDA indices calculation

In this thesis ultrasound volumes were calculated using one of two methods. When the Combison 530 3D was used volume was measured by delineating the whole of the object of interest in a number of parallel longitudinal sections 1 -2 mm apart. The volume was then calculated automatically by the built in program. The majority of volume measurements were carried out using the Voluson 730 or E8 with the VOCAL imaging program. When using VOCAL the longitudinal image (A) was used as reference and the antero – posterior axis was used for rotation (Figure 5). A set of callipers was placed at the lower and upper aspects of the object of interest so that rotation between these two points would include the entirety of the object. A rotational step of 9° was used and the object of interest was traced through each step until completion of an 180° rotation. As discussed in the introduction, the validity, reliability and reproducibility of these methods to measure endometrial volume in benign and pathological processes has been demonstrated previously (Gruboeck et al. 1996; Raine-Fenning et al. 2002; Merce et al. 2006; Cheong et al. 2009; Opolskiene et al. 2010).

1.3.5 Off - line 3D – SIS analysis

When 3D – SIS volumes were analysed a variety of measurements were made. We used the longitudinal image (A) as reference. We then adjusted the sectional plane until the plane of maximum protrusion of the fibroid was identified (Figures 6 and 7). The endometrial – myometrial junction was subjectively identified and a projected line was drawn through the submucous fibroid demarcating intracavitary and intramural components. The section of the fibroid protruding into the cavity (A) and the intramural component (B) were both measured (Figure 8). Care was taken not to include the overlying myometrium. A protrusion ratio expressed as a percentage $A/(A + B) \times 100$ was then calculated to describe the degree of fibroid protrusion into the uterine cavity. The fibroid diameter was determined as the sum of $A + B$. The fibroid was then classified according to the European Society of Hysteroscopy Classification of Submucous Fibroids (Wamsteker et al. 1993) as Type 0 (fibroid polyp), Type 1 (<50% confined in the myometrium or protrusion ratio >50%) or Type 2 ($\geq 50\%$ confined in the myometrium or protrusion ratio $\leq 50\%$). We also measured the distance between the lowermost part of fibroid and the internal cervical os. The volume of the fibroid was then calculated using VOCAL and the same method described above. The fibroid was then classified according to the European Society of Hysteroscopy Classification of Submucous Fibroids (Wamsteker, 1993) as Type 0 (fibroid polyp), Type I (<50% contained within the myometrium) or Type II (>50% contained within the myometrium). All variables were recorded in a computerised database (MS Excel 2003).

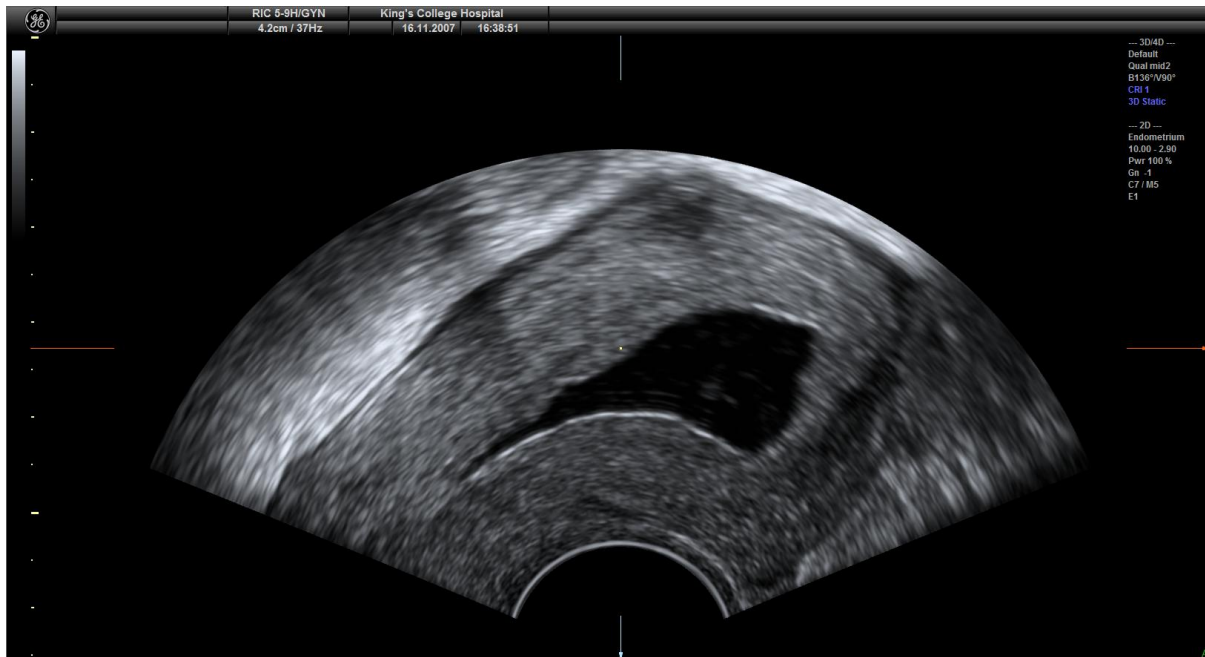


Figure 6 Image capture demonstrating the reference image in 3D - SIS off line analysis

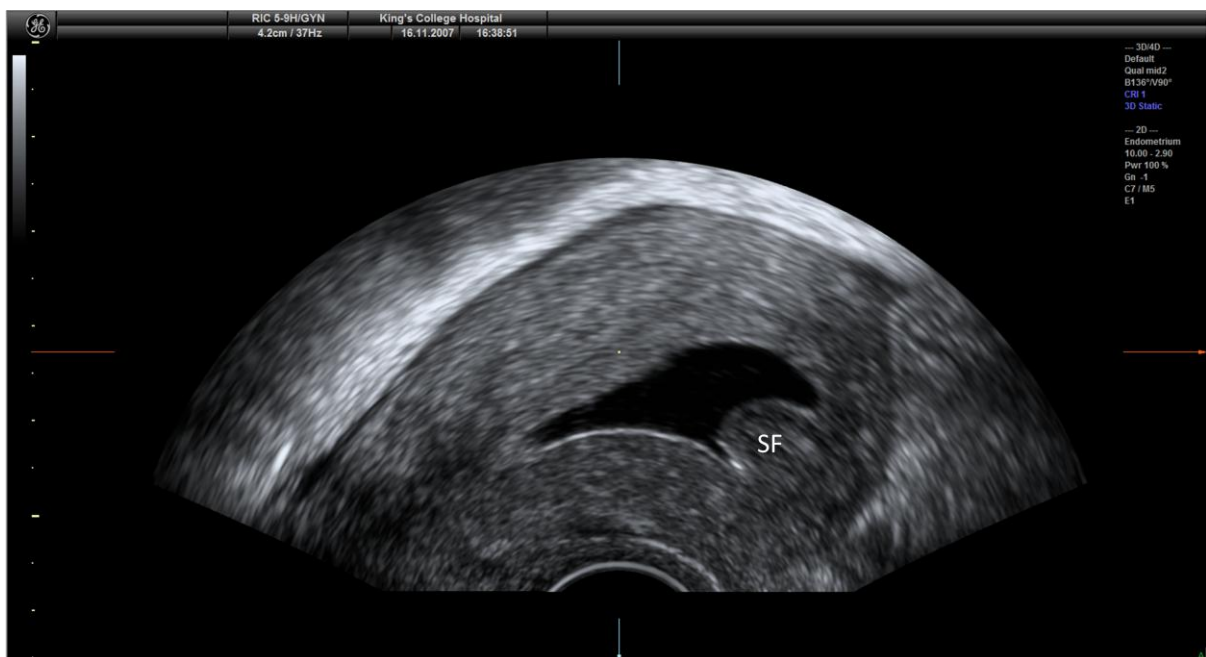


Figure 7 Image capture of 3D - SIS off line analysis demonstrating the presence of a submucous fibroid (SF) after adjustment of the sectional plane

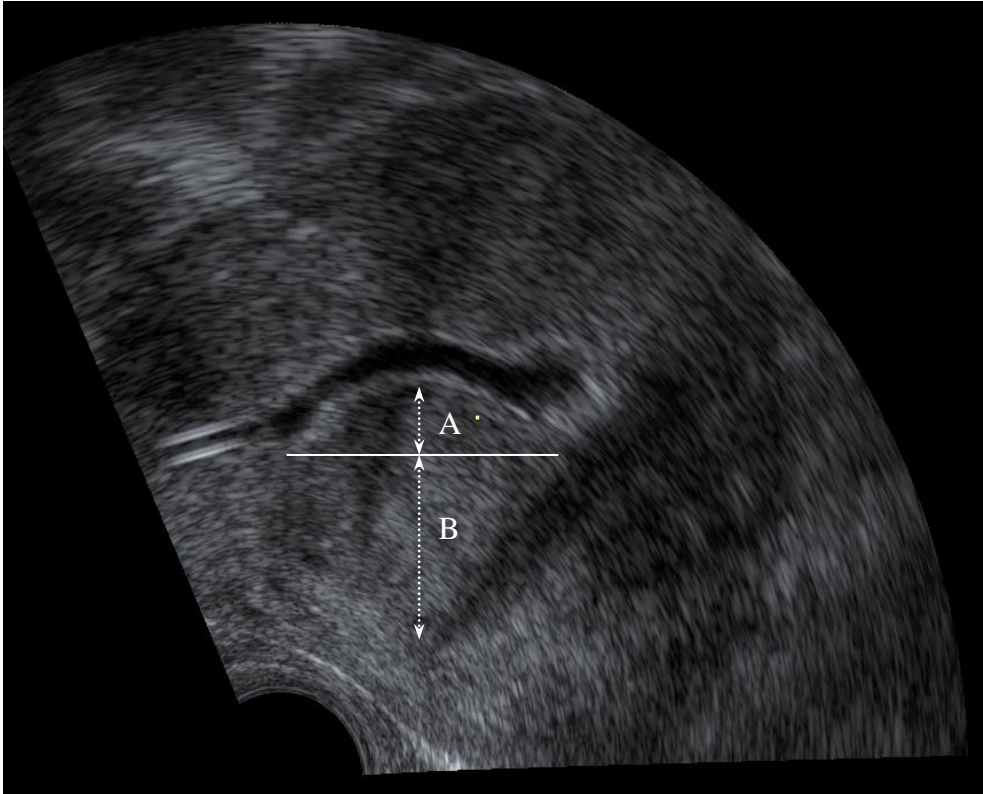


Figure 8 Image illustrating the measurement of the intracavitary (A) and intramural (B) portion of a Type 2 submucous fibroid during off line analysis of 3D - SIS

1.4 Endometrial sampling

In postmenopausal women with endometrial thickness $\geq 5\text{mm}$ endometrial sampling was performed after either 3D or 3D – PDA ultrasound volumes were acquired and stored for analysis. The endometrium was assessed subjectively by gray scale 2D and power Doppler for the presence of a focal or global lesion. In women with a global, uniform lesion a Pipelle (Prodimed, Neuillyen-Thelle, France) biopsy was performed in the clinic. In cases where a focal pathology was suspected, outpatient or day case hysteroscopy was arranged.

1.5 Transcervical resection of fibroid

TCRF was performed under general anaesthesia with a rigid 30° resectoscope with bipolar loop wire electrodes (Storz Endoscopy, Germany). Aseptic technique was observed throughout the procedure. Normal saline was used to distend the uterine cavity. Infusion pressure was elevated by a pneumatic cuff under manometric control to 100–120 mmHg. A high intensity cold light source and fibre optic cable were used to illuminate the uterine cavity. The procedure was monitored using a single chip video camera and the image was displayed on a monitor visible to the operator. The approximate size of the fibroid was determined by visualization of the fibroid and comparison with the length of the hysteroscope. The protrusion into the cavity was determined by visualization of the angle between the fibroid and the uterine wall (Figure 9A). For all fibroids an attempt was made to resect them completely. Throughout the operation the balance between infused and collected fluid was monitored. Any fluid deficit was recorded at the end of the procedure. The time lapsed for each procedure was recorded as the time from the initial application of the tenaculum to time of completion of the resection. Blood loss was estimated by subjective evaluation by the nurse in charge of the theatre. The aim of the procedure was to resect completely the fibroid(s) without risking perforation of the myometrium or fluid overload. Accordingly the stopping rules of the operation were designed to accommodate this aim without risking injury to the patient. The procedure was stopped when all the fibroid had been removed. The procedure was also stopped when the resection had gone approximately 1 cm deep into the myometrium and the operator felt that it would be unsafe to proceed with resection. The depth of resection was measured from the endometrial surface to the deepest point of the space in the myometrium from where the fibroid had been removed by

comparing the depth to the loop (Figure 9C). Fluid balance was reported throughout the procedure by the nurse in charge and the procedure was stopped if the fluid deficit exceeded 1.5L or it was not possible to visualise the uterine cavity due to excessive bleeding.

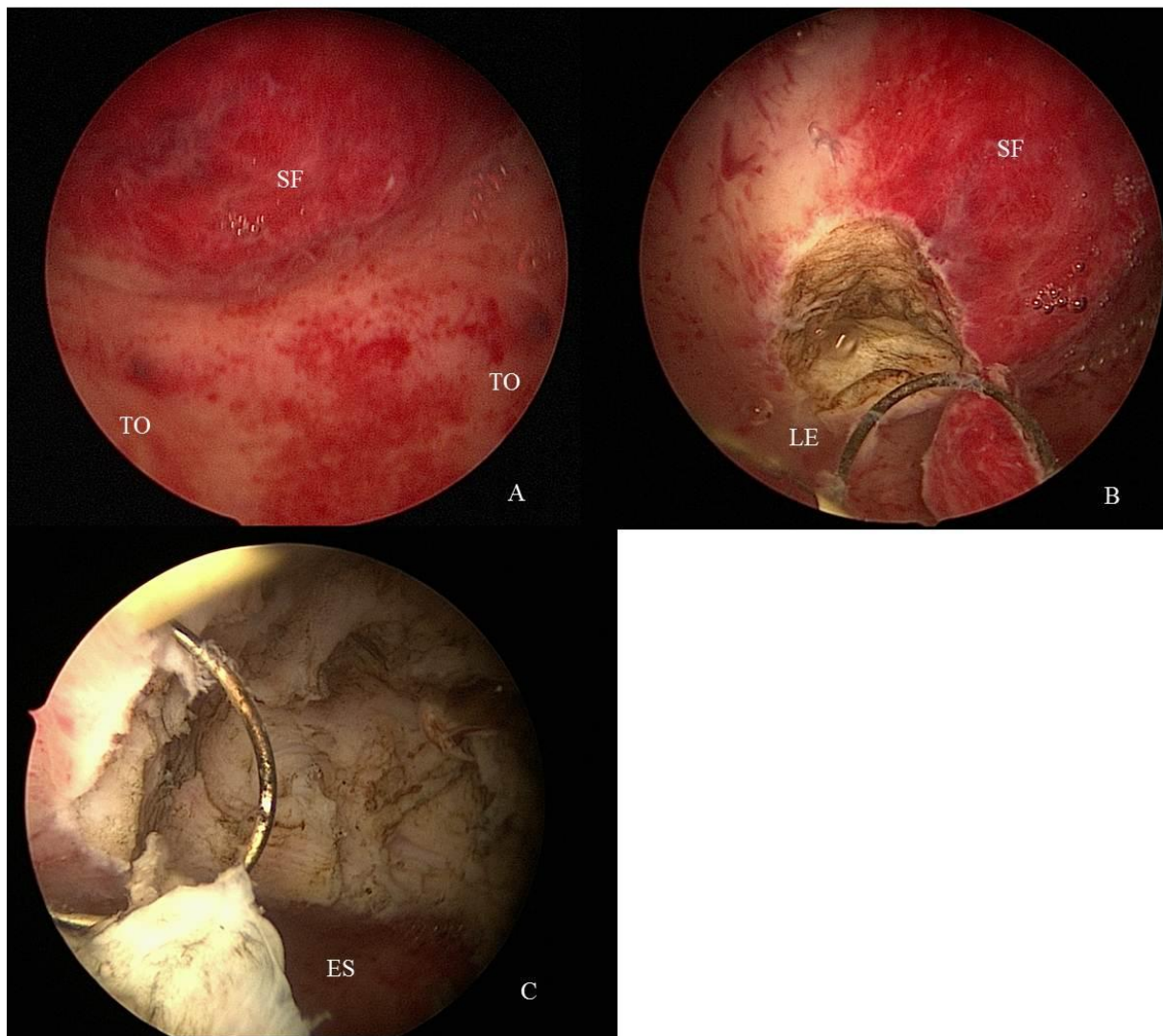


Figure 9 TCRF (A) Type II submucous fibroid (SF) protruding from the anterior myometrium. Both tubal ostia (TO) are seen. Note difficulty in objective estimation of fibroid size protrusion. (B) First incision by loop electrode (LE) (C) The majority of the fibroid has been removed resection has gone below the endometrial surface (ES)

1.6 Statistical analysis

Data was collected and stored in an electronic database (Microsoft Excel 2003). The Statistical Package for Social Sciences 16.0 – 20.0 (Statistical Analysis Systems, Chicago Illinois) was used for data analysis. Throughout this thesis, the Kolmogorov – Smirnov test was used to test for normal distribution. Dichotomous variables are expressed as percentage (95% CI), non - normally distributed variables as median (IQR) and normally distributed variables as mean (SD). We used the χ^2 test to compare proportions, the Mann – Whitney test to compare the medians of two non - normally distributed variables, the Kruskal – Wallis test to compare 3 or more medians and Student’s t test to compare the means of normally distributed variables. For multivariate analysis of dichotomous dependent variables forward conditional logistic regression was used. When categorical variables were included as independent variables in logistic regression “dummy coding” was used. To evaluate the diagnostic performance of a test or logistic regression model ROC curves were plotted and the AUC was calculated. Details of individual tests are documented in relevant chapters. Statistical significance level was $p < 0.05$.

1.7 Ethical Committee approval

The Local Research and Ethics Committee at King’s College Hospital granted approval for the randomized controlled trial. This study was registered on the Current Controlled Trials Website: <http://www.controlled-trials.com/mrct/trial/662485/ISRCTN06560767>.

No treatment decisions were based on 3D endometrial volume and 3D – PDA analysis. We were advised that these studies did not require a formal ethical committee assessment and approval.

Part III Results

[1] Natural history of fibroids

1.1 Background

Fibroids are the commonest benign uterine tumours and it is estimated that they occur in 20 to 40% of women during their reproductive years (Wallach et al. 2004; Ryan et al. 2005). They can cause a wide range of clinical symptoms such as heavy menstrual periods, pressure symptoms to surrounding organs and fertility problems (Parker 2007). As a result, surgery for uterine fibroids is common, and in both the UK and USA fibroids are a leading indication for hysterectomy (Vessey et al. 1992; Marshall et al. 1997). However, in many women, uterine fibroids are found incidentally on routine gynaecological examination or if pelvic imaging is done for unrelated symptoms. Whether fibroids are symptomatic or not depends primarily on their size and on their position in relation to the uterine cavity (DeWaay et al. 2002). Although the definitive management of gynaecological symptoms attributable to uterine fibroids is surgical removal, many women decline surgery or prefer to pursue medical management. The natural history of fibroids is poorly understood with only two longitudinal studies published to date (DeWaay et al. 2002; Peddada et al. 2008). This makes it difficult to advise asymptomatic women with fibroids on the risk of developing clinical symptoms in the future. It is well known however that fibroids are sensitive to circulating oestrogens (Blake 2007), which will either cause them to grow or to maintain their size. It is less clear whether the growth of fibroids is affected by other factors than ovarian steroid hormones. The aim of this study was to describe the natural history of uterine fibroids in pre-menopausal women

and to identify demographic and morphological features, which may influence their growth rate.

1.2 Methods

This was a retrospective study. We searched our ultrasound clinic database (PIA Fetal Database, version 3.23, Viewpoint Bildverarbeitung GmbH, Munich, Germany) to identify all women diagnosed with uterine fibroids between July 1997 and July 2006. We only included women who had been examined at least twice by a single expert ultrasound operator with a minimum interval of eight months between the examinations. In order to establish a stable hormonal environment we established the following inclusion criteria: age 25-45 at the time of initial examination, regular menstrual cycles lasting between 21 and 35 days, no use of any form of hormonal contraception or any other medication with possible effect on ovarian hormone production and no previous surgery for fibroids. We excluded all women who fell pregnant during the interval between examinations; those who developed menstrual irregularities or had surgery for fibroids.

1.3 Statistical analysis

Only the largest fibroid for each woman was included in our analysis in order to ensure consistency of measurement during follow up. We used the measurements as recorded in our database at the time of examination to calculate the mean diameter as $d_{\text{mean}} = (d_1 + d_2 + d_3)/3$. We calculated the volume of the largest fibroid using the formula for the volume of a sphere ($V = 1/6 * \pi * d^3$). Fibroid growth rate (r) was calculated as $r = (V_{t2} - V_{t1}) / V_{t1} * 100$, yearly growth rate (r_y) was calculated as $r_y = r / \text{interval months} * 12$. It has been previously shown that the validity and reproducibility of fibroid diameter measurement by ultrasound is good

with an average difference between observers of 0.391 mm (Dueholm et al. 2002; Lee et al. 2006). In order to account for intraobserver variability and given that the vast majority of fibroids included were larger than 10mm diameter we defined spontaneous regression as a 5% decrease in volume over one year. This is likely to reflect true changes in fibroid size rather than intraobserver variability. We categorized women to under 35 years of age or 35 or more years of age to maintain consistency with published work (Peddada 2008). The normal distribution for fibroid volume and yearly percentile growth in fibroid volume was rejected ($p=0.001$) and so non-parametric tests were used. The variables were expressed as median (interquartile range). The yearly percentile growth in fibroid volume was transformed using Box – Cox transformation to a normal distribution. Multivariate linear regression was used to detect predictive variables for yearly percentile fibroid growth rate. Variables included in the model were age, parity (categorical; 0, ≥ 1) fibroid number (categorical; 0, ≥ 1), fibroid position (categorical; subserous, intramural, submucous) and fibroid size (categorical; $<20\text{mm}$, 20 – 50mm, $>50\text{mm}$).

1.4 Results

178 women between 25 and 45 years of age who underwent ≥ 2 examinations by a single, expert operator were identified. 21 women were excluded because of the use of hormonal contraception during follow up, 16 because the interval between the initial and follow up scan was <8 months, 9 became pregnant during follow up, 5 developed irregular menstrual cycles during follow up, 2 underwent TCRF, 2 were commenced on GnRH analogues and 1 woman was started on tamoxifen. The indications for examination in 122 women who were included in the final data analysis are presented in (Table 6). The median age at the initial scan was 40 years (range 27 – 45years) and 72/122 (59%) women were nulliparous. The median

examination interval between the initial and final examination was 21.5 months (range 8 – 90 months) and 74/122 (60.7%) women had multiple fibroids.

Women who presented with a suspicion of fibroids on clinical examination or menorrhagia had significantly larger fibroids (median 13.8 cm³, IQR 2.3 – 61.9) compared to women presenting with pain or other symptoms (median 1.6 cm³, IQR 0.5 – 27.4) ($p < 0.001$)

During the study period the median volume of the largest fibroid increased from 9.9 cm³ (IQR 1.0 – 42.8) at the initial examination to 12.0 cm³ (IQR 2.4 – 85.2 cm³) ($p < 0.001$) at the final examination. This corresponded to a median 35.2% (IQR 0.0 – 107.9) yearly increase in the volume of the largest fibroid. Fibroid growth rate demonstrated considerable variability with 26/122 (21.3%, 95% CI 14.4 – 28.6) fibroids showing evidence of spontaneous regression (Figure 9). There was no significant difference in fibroid growth rate between women presenting with menorrhagia or a suspicion of fibroids on clinical examination compared to women with other initial complaints ($p = 0.162$). In women ≤ 35 years of age the volume of the largest fibroid grew by 69.1% (8.3 – 185.1%) in one year compared to women > 35 years of age in whom the volume of the largest fibroid grew by a median 29.8% (0.0 – 78.7%) ($p = 0.113$) in one year (Figure 12). There was no significant difference in fibroid growth rate between nulliparous and parous women ($p = 0.827$) or between women with a single and multiple tumours ($p = 0.444$). Fibroid position was significantly associated with fibroid growth rate. Intramural fibroids grew in volume by a median 53.2% (IQR 11.2 – 217.0) in one year compared with subserous fibroids which grew by a median 25.1% (IQR 1.1 – 87.1) in one year and submucous fibroids which grew by a median 22.8% (IQR -11.7 – 48.3) ($p = 0.012$) (Figure 13). Initial fibroid size was also significantly associated with growth rate. Small fibroids (< 20 mm) and large fibroids (> 50 mm) demonstrated the fastest growth

and grew by a median 51.3% (IQR 9.3 – 210.3) and 40.7% (IQR 14.1 – 67.0) in one year respectively. Intermediate fibroids (20 – 50mm) demonstrated slower growth and grew by a median 16.8% (IQR -7.4 – 63.0) in one year ($p=0.007$) (Figure 14 and Figure 15). Multivariate analysis demonstrated that initial fibroid size is a significant independent predictor for fibroid growth rate whilst the association between fibroid position and growth rate disappears once we adjust for initial fibroid size (Table 7).

Figure 10 Distribution of the growth rate of the largest fibroid during the study period with superimposed normal distribution curve (n=122)

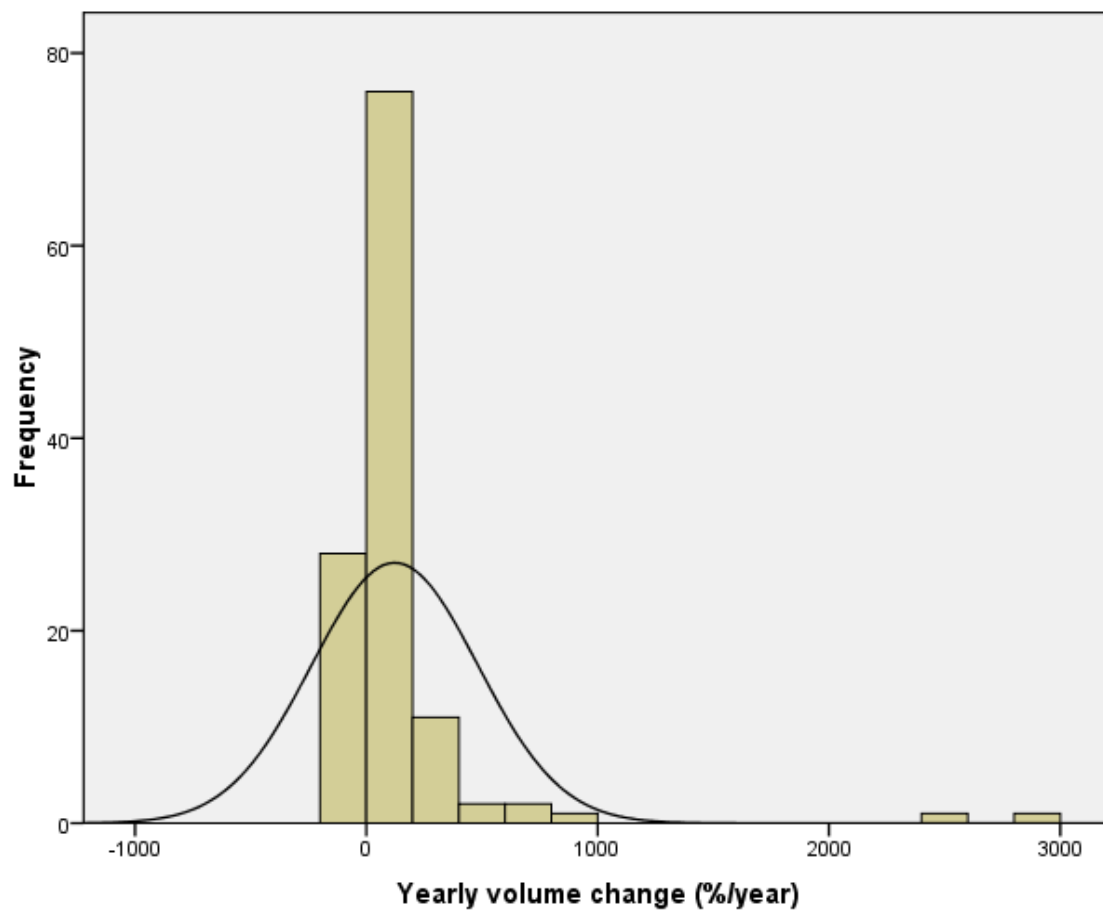


Figure 11 Distribution of the growth rate of the largest fibroid during study period after Box - Cox transformation to normal distribution (n=122)

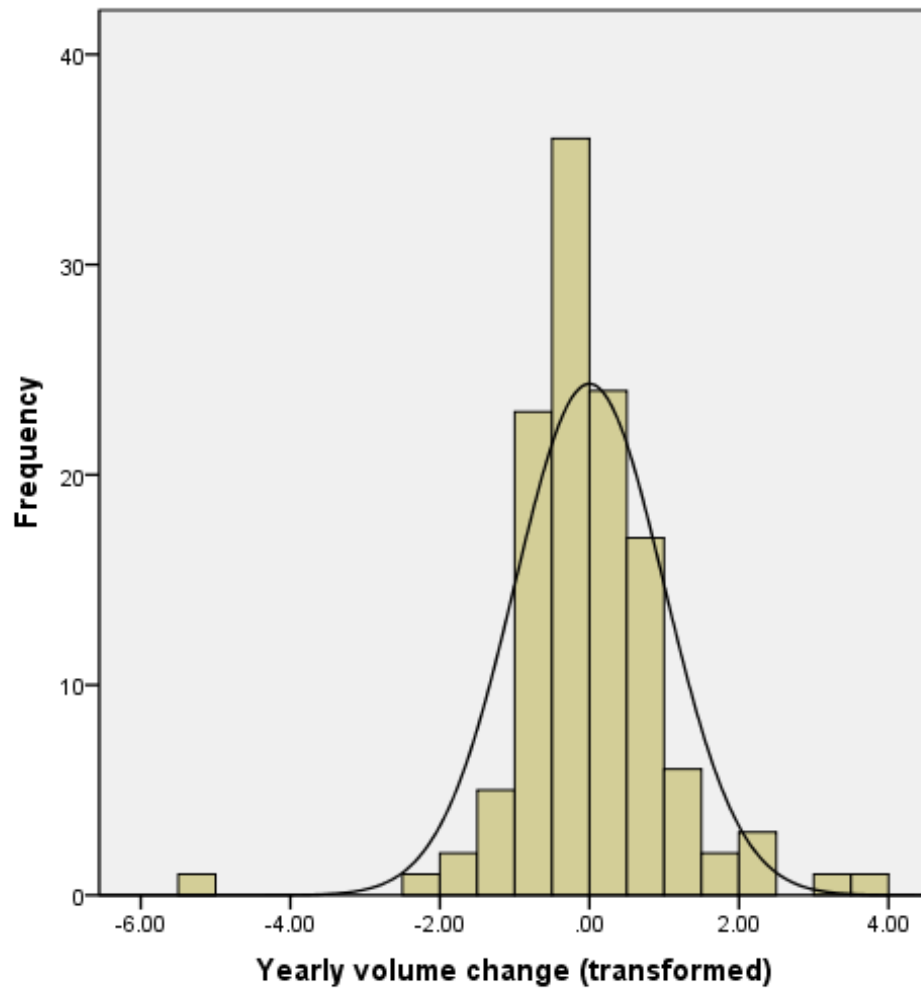


Figure 12 Boxplot comparing the median growth rate (IQR) in the volume of the largest fibroid between women aged ≤ 35 (n=26) and those >35 years (n=96) (logarithmic scale)

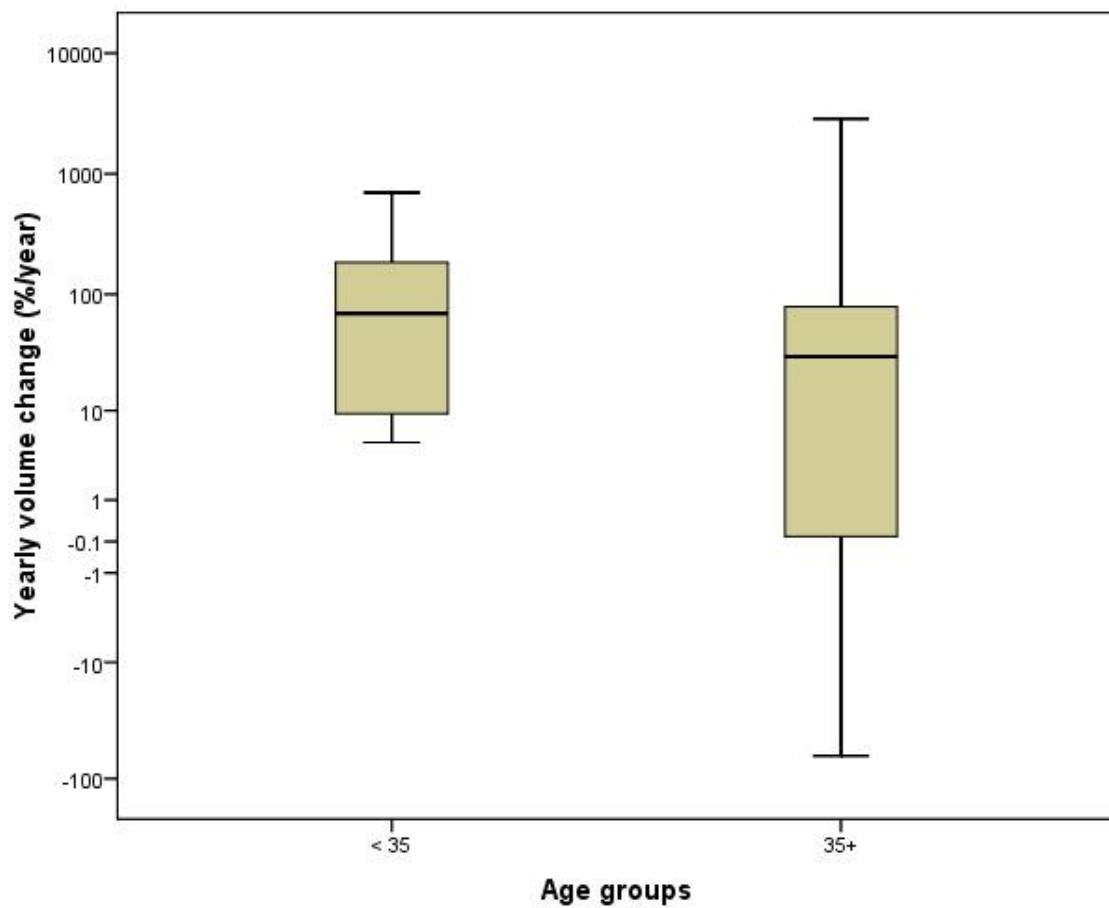


Figure 13 Boxplot comparing the median growth rate (IQR) in the volume of the largest fibroid between fibroids that were submucous (n=23), intramural (n=42) and subserous (n=57)

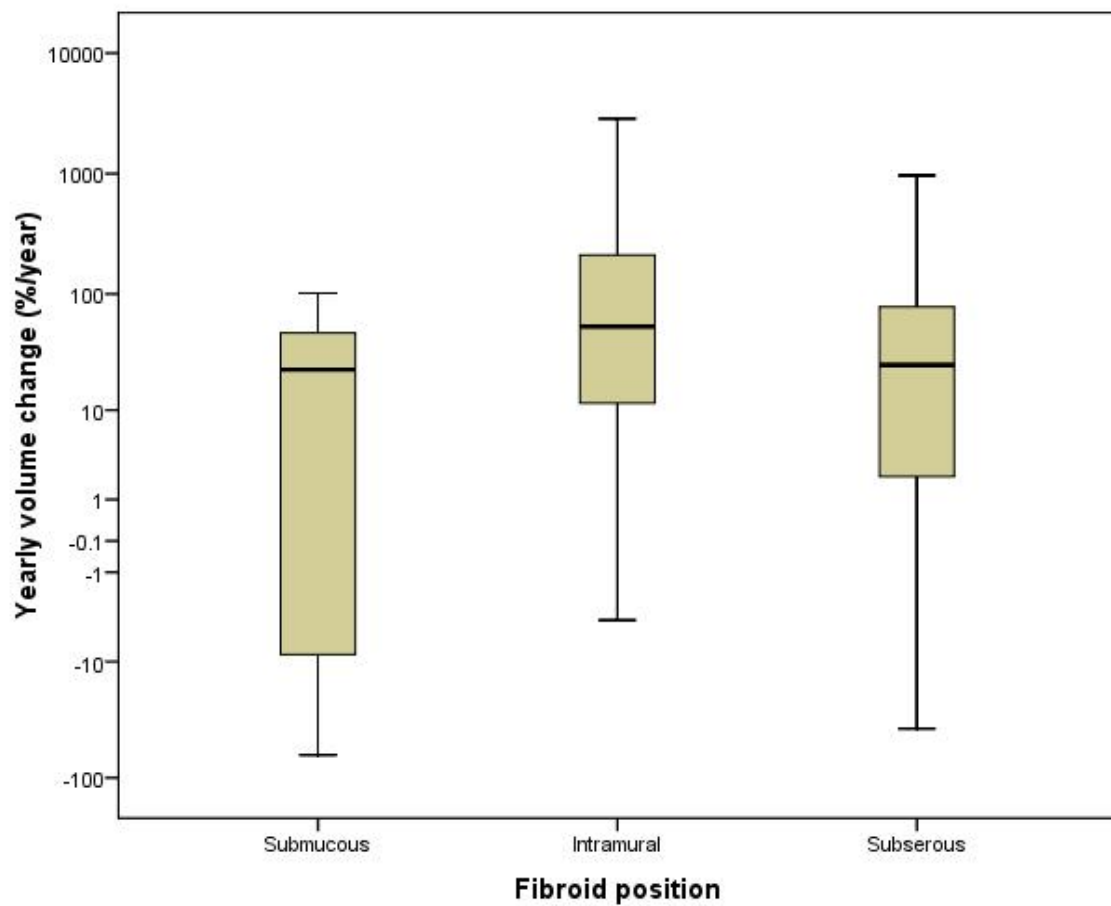


Figure 14 Boxplot comparing the median growth rate (IQR) in the volume of the largest fibroid between fibroids that were <20mm in diameter at presentation (n=48), 20 – 50mm in diameter at presentation (n= 51) and >50 mm in diameter at presentation (n=23)

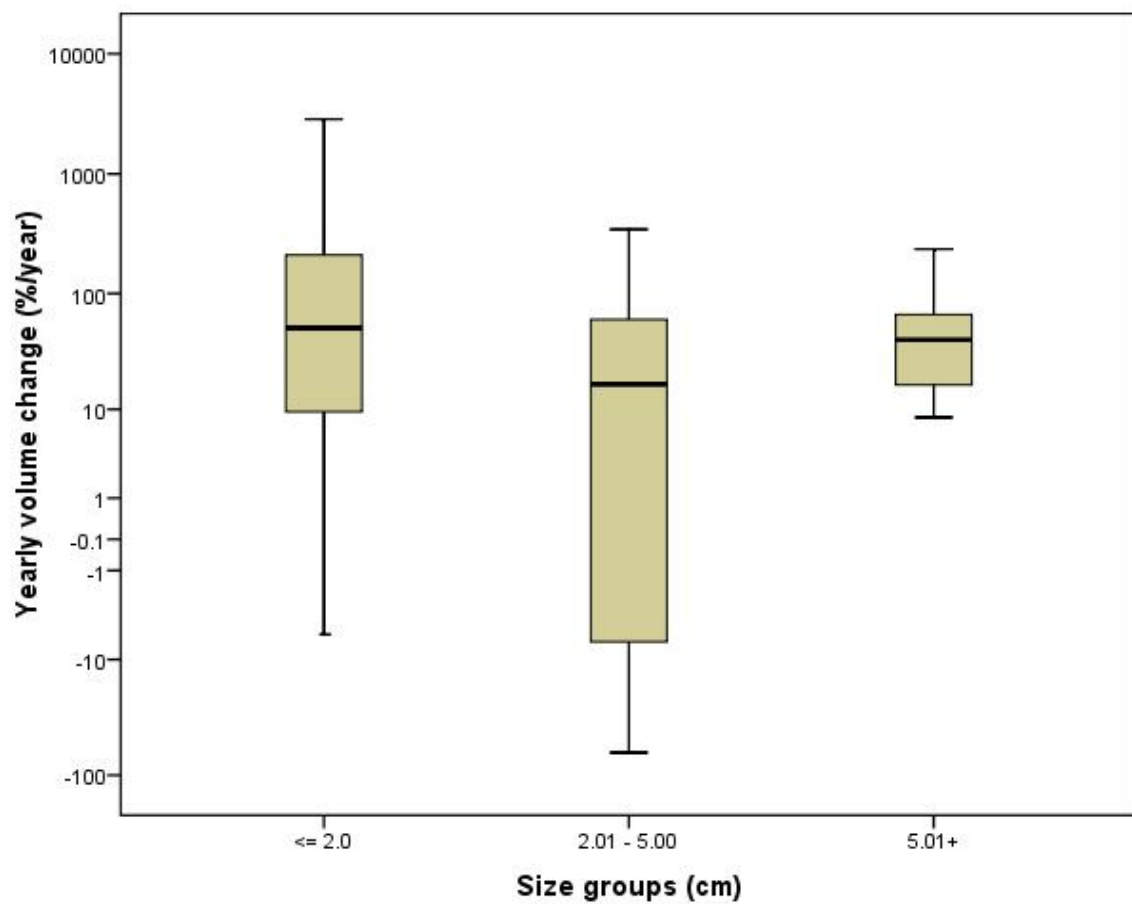


Figure 15 Scatterplot of change in diameter against fibroid diameter at presentation with superimposed fitted curves. Linear plot ($R^2=0.01$, $p=0.245$), cubic curve ($R^2=0.18$, $p=0.001$)

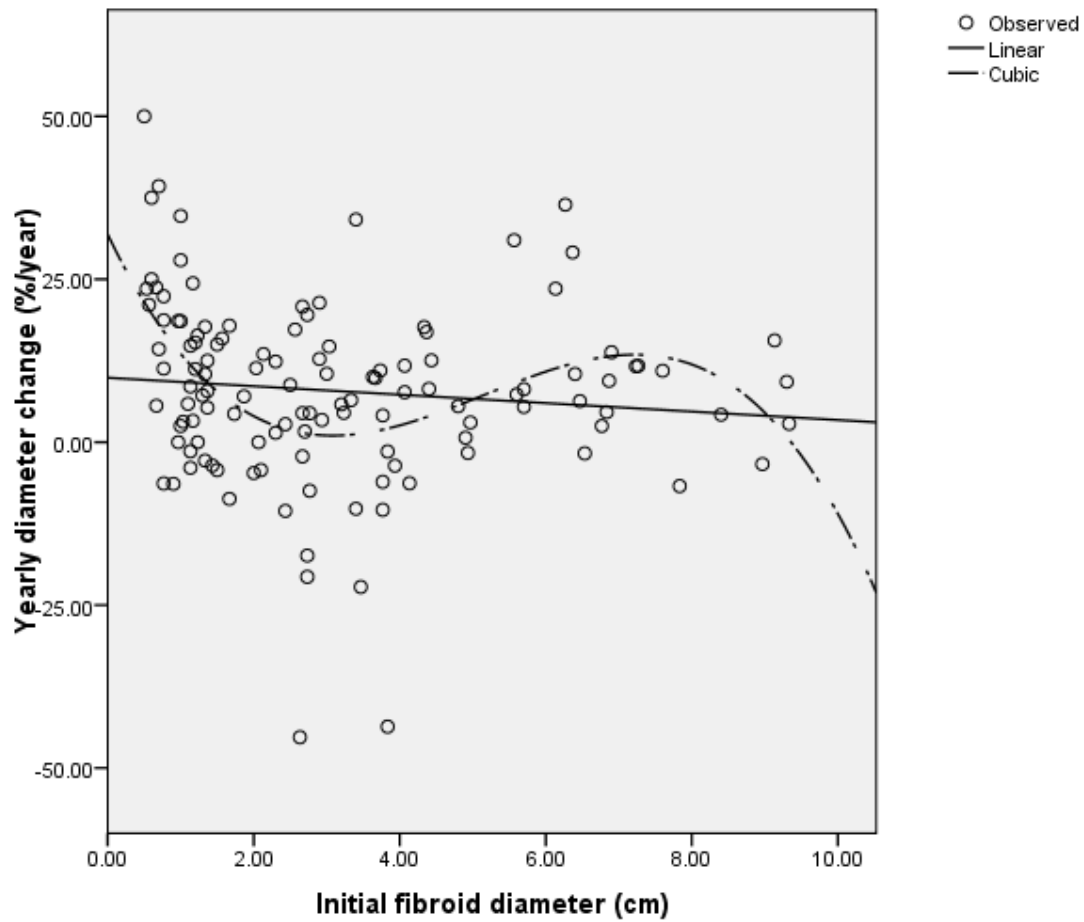


Table 6 List of indication for ultrasound examination in women diagnosed with fibroids and included into the study (n=122)

Indication for assessment	n	(%)
Heavy periods	43	(35)
Suspected fibroids on clinical examination	30	(25)
Pelvic pain	29	(24)
Suspected ovarian cyst	8	(6)
Infertility	4	(3)
Screening for ovarian cancer	2	(2)
Urinary frequency	2	(2)
Other	4	(3)
Total	122	(100)

Table 7 Results of multivariate analysis of the prediction of fibroid growth including fibroid size at presentation and fibroid position ($R^2=0.123$)

	Beta	t	R^2
Step 1			
(Constant)		2.882	.118
20 – 50 mm	-.758	-3.980	
> 50 mm	-.409	-1.703	
Step 2			
(Constant)	.209	1.066	.162
20 – 50 mm	-.520	-2.426	
>50mm	-.189	-.731	
Submucous	-.352	-1.516	
Intramural	.321	1.499	

Dependent variable: yearly fibroid growth

[2] Three dimensional saline infusion sonohysterography for the preoperative prediction of submucous fibroid resection

2.1 Background

Submucous fibroids are a recognised cause of heavy and irregular menstrual periods (Clevenger-Hoeft et al. 1999). They have also been identified as a potential cause of sub-fertility and early pregnancy failure (Pritts 2001). In the past, the only available surgical treatment for fibroids was open myomectomy. In recent years transcervical resection has been introduced into clinical practice. This procedure avoids the risks associated with open surgery, enables removal of submucous fibroids with minimal risk of pelvic adhesions and has rapid post operative recovery. However, the procedure is not always successful and some fibroids cannot be completely removed (Emanuel et al. 1999). In an attempt to assess the suitability of submucous fibroids for resection, a classification system which divides them into 3 groups depending on the degree of protrusion into the cavity has been developed; Type 0 fibroids are exclusively in the cavity, Type 1 are more than 50% intracavitary while Type 2 are less than 50% intracavitary. Even though this classification is widely adopted in clinical practice it is not very accurate in predicting the success of hysteroscopic resection. Previous studies have shown that the probability of Type 1 and Type 2 fibroids to be completely resected is 60% and 50% respectively, meaning that the system does not readily differentiate

between fibroids that will and those that will not be completely resected at a single procedure (Wamsteker et al. 1993) . It is clear that other factors play a role in determining the success of surgery but these have not been explored. This may be a consequence of the widespread use of diagnostic hysteroscopy to diagnose and assess the suitability of submucous fibroids for resection. Hysteroscopy is a subjective method and its reproducibility to determine the percentage of fibroid protrusion in the cavity has not been clearly established. In addition, it is almost impossible to accurately measure the size of fibroids on hysteroscopy which may explain why size is not included in the current classification (Wamsteker et al. 1993; Emanuel et al. 1997). One may reasonably expect that when a minimally invasive approach is used larger fibroids will be more difficult to remove than smaller ones. Ultrasound offers a number of advantages over hysteroscopy for the assessment of submucous fibroids; it is easy and quick to perform, does not require anaesthetic and allows objective estimation of fibroid size. Ultrasound has been used for the assessment of submucous fibroids and recently it has been shown that 3D – SIS is reproducible and equivalent to diagnostic hysteroscopy (Lee et al. 2006). However, compared to hysteroscopy, 3D – SIS provides a wealth of information which has not yet been evaluated for its utility in the preoperative assessment of submucous fibroids. The aim of this study is to examine which of these variables are significant predictors of complete fibroid resection at operative hysteroscopy and to devise a classification based on these factors.

2.2 Methods

This was a prospective study performed at the EPAGU at KCH. The inclusion criteria were: symptoms of heavy and irregular periods and diagnosis of a submucous fibroid on 2D transvaginal ultrasound. Women who met the inclusion criteria were referred for 3D – SIS.

All 3D – SIS were performed by a single operator using the methodology described in the methods chapter. All ultrasound volumes were stored and analysed off – line using VOCAL. All women found to have a submucous fibroid were referred for TCRF. TCRF was performed as described in the method section by a single experienced operator.

2.3 Statistical analysis

The aim of the study was to assess the value of various variables for the prediction of complete resection of submucous fibroids at TCRF. We compared the age, parity, the median maximum fibroid diameter, the median size of the intramural component, the median distance from the internal cervical os and the mean protrusion ratio between women that had complete and those that had incomplete fibroid resection at TCRF. We then split the data into two sets training and a testing set. We programmed the statistical package to randomly select 60% of cases which produced the training set on which the logistic regression was carried out. The remainder of the cases were used as a testing set on which the derived regression equation was cross – validated. The randomly selected training set contained 39 cases consisting of 22 complete and 17 incomplete resections. The testing set contained 22 complete and 6 incomplete resections. There was no significant difference in all the variables between the training and testing set. We performed forward conditional multivariate logistic regression analysis with completeness of resection as independent variable and all other variables, including parity (nulliparous [0] vs. parous [1]), fibroid position (anterior – posterior [1] vs. fundal [0]) and number of fibroids (single [1] vs. multiple [0]) as dependent variables. For each variable included in the logistic regression we plotted ROC curves and selected the optimal cut – off. For all tests a two – tailed p value of less than 0.05 was considered significant.

2.4 Results

Between August 2006 and February 2008 80 women with symptomatic submucous uterine fibroids were assessed with 3D – SIS and were provisionally booked for TCRF. 19 women were not operated or did not have a complete dataset. 13/19 (68.4%, 95% CI 47.5 – 89.3) women were lost to follow up, 3/19 (15.8%, 95% CI 0.0 – 32.2) did not attend for their operation, 3/19 (15.8%, 95% CI 0.0 – 32.2) opted for abdominal myomectomy. Out of 61 women who were included in the data analysis 55 had a single submucous fibroid and 6 women had two fibroids each. The total number of fibroids operated on was 67. 5/67 (7.5% 95% CI 1.2 – 13.8) fibroids were Type 0, 42/67 62.7% (95% CI 51.1 – 74.3) were Type 1 and 19/67 28.4% (95% CI 17.6 – 39.2) were Type 2.

49/67 (73.1%, 95% CI 62.5 – 83.7) fibroids were completely resected at TCRF. There were no significant differences in the mean women's age ($p=0.435$), the proportion of parous women ($p=0.141$), the proportion of women with multiple fibroids ($p=0.281$) and the median distance between the distal part of the fibroid and the internal cervical os ($p=0.472$) between women who had complete and incomplete resection. The mean protrusion ratio, the median fibroid diameter and the median size of the intramural component (B) were all significantly different in women with complete and incomplete resections (Table 8).

The results of the multivariate analysis for the training set ($n=39$) are presented in Table 9. Parity, the size of the fibroid's intramural component and the fibroid diameter were significantly associated with complete fibroid resection.

In the training set the model had an AUC of 0.975 (SE 0.039) with 96.3% (95% CI, 81.0 – 99.9) sensitivity and 91.7% (95% CI 61.5 – 99.8) specificity based on a cut - off of 35.9%

calculated probability. When this model was applied to testing set (n=28) the AUC was 0.864 (SE 0.090) with 86.4% (95% CI 65.1 – 97.1) sensitivity and 83.3% (95% CI 35.9 – 99.6) specificity based on a cut – off of 9.3% calculated probability ($p>0.05$) (Figure 16). We also plotted ROC curves for degree of protrusion, fibroid diameter and size of the intramural component for the testing set (Table 10).

During the study period one woman with incomplete fibroid resection had significant bleeding that necessitated insertion of a Foley catheter to achieve haemostasis and needed overnight admission. Another 8/18 (44.4%, 95% CI 21.5 – 67.4) women with incomplete resection had the procedure abandoned because the fluid deficit exceeded 1.5 L. In the remaining 9/18 (50%, 95% CI 26.9 – 73.1) women the procedure was abandoned when resection had proceeded more than 1cm depth into the myometrium. One woman who had complete resection of her fibroid had fluid overload that necessitated diuretic treatment.

Table 8 Univariate analysis of the characteristics of women (n=61) and their fibroids (n=67) with complete (n=49) and incomplete resection (n=18) at TCRF

	Complete resection (n=49)	Incomplete resection (n=18)	P
Age (years, (mean, SD))	42 (5.6)	39 (7.5)	0.435
Nulliparous women (n (%; 95% CI))	19 (38.8; 25.1 – 52.4)	3 (16.7 (0.0 – 33.7)	0.141
Multiple fibroids (n (%; 95% CI))	7 (14.3; 4.5 – 24.1)	5 (27.8; 7.1 – 48.5)	0.281
Mean protrusion ratio (% (SD))	67.8 (14.5)	47.0 (17.8)	<0.001
Median diameter (mm (IQR))	24.0 (19.0 – 30.5)	45.0 (28.75 – 51.25)	<0.001
Median size of intramural component, (mm (IQR))	8.0 (4.0 – 11.5)	16.5 (12.5 - 29.25)	<0.001
Median distance to cervical os (mm (IQR))	15.5 (9.0 – 20.0)	14.0 (8.0 – 24.0)	0.472
Fundal location (n (%; 95% CI))	15 (30.6; 7.8 – 43.5)	4 (22.2 ; 3.0 – 41.4)	0.559

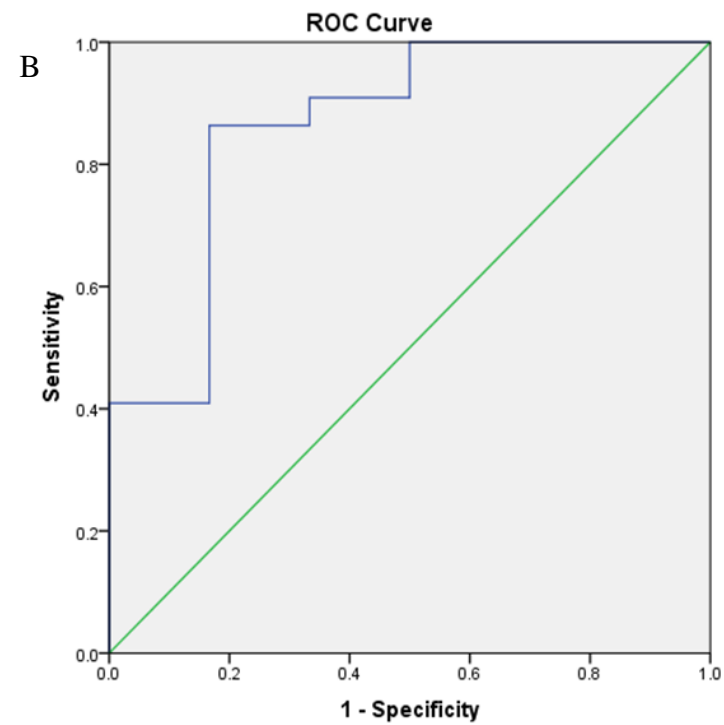
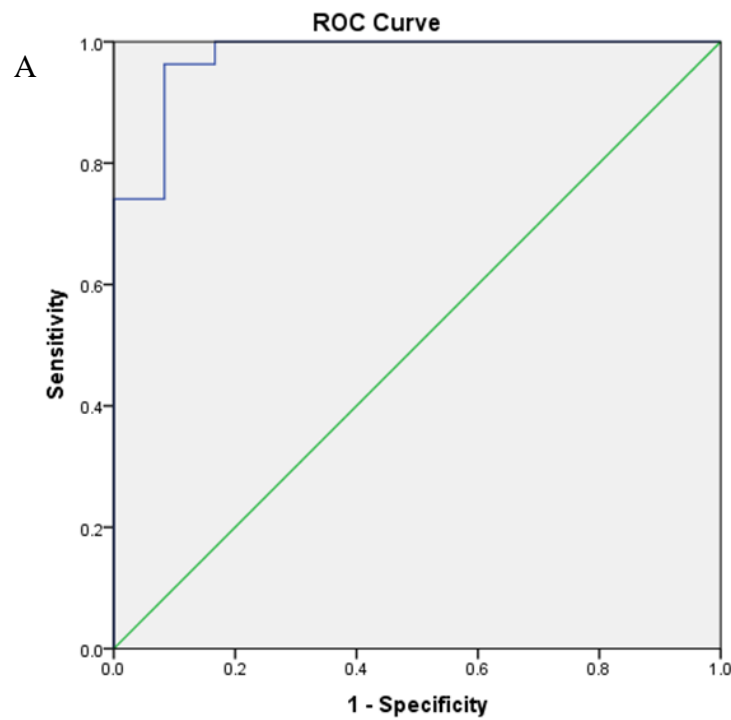
Table 9 Results of multivariate analysis to indentify significant predictors of completeness of fibroid resection at TCRF (training set, n=39)

	OR (95% CI)	P
Age	-	0.418
Parity	0.002 (0.0 – 0.035)	0.035
Size of intramural component	0.511 (0.277 – 0.943)	0.032
Fibroid diameter, mm	0.843 (0.655 – 1.0)	0.05
Degree of protrusion, % (\pm SD)	-	0.132
Distance from internal cervical os, mm	-	0.597
Fibroid position	-	0.496
Fibroid multiplicity	-	0.148

Table 10 ROC curve analysis for the application of the logistic regression model, the degree of protrusion, the fibroid diameter and the intramural depth on the testing set (n=28)

	AUC (SE)	Cut – off value	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Logistic regression model	0.864 (0.090)	>9.3%	86.4 (65.1 – 97.1)	83.3 (35.9 – 99.6)
Degree of protrusion	0.777 (0.114)	43% protrusion	86.4 (65.1 – 97.1)	66.7 (22.3 – 95.7)
Fibroid diameter	0.867 (0.070)	38.0mm	86.4 (65.1 – 97.1)	83.3 (35.9 – 99.6)
Size of intramural component	0.833 (0.090)	10mm	59.1 (36.4 – 79.3)	100 (54.1 – 100)

Figure 16 ROC curve for the calculated probability from the logistic regression model for the training (n=39) (A) and the testing (n=28) (B) sets. The AUC were 0.975 and 0.864 respectively.



[3] Randomised controlled trial for the use of GnRH before transcervical resection of fibroid

3.1 Background

As discussed in the previous study, submucous fibroids can be treated effectively by hysteroscopic resection. However in order to obtain good views of the endometrial cavity a fluid medium, such as glycine or saline, is necessary to achieve adequate distension. Some of this fluid is absorbed into the intravascular space and if the operation is prolonged, absorption of excess fluid can lead to considerable complications such as pulmonary and cerebral oedema. Severe cases may need intensive treatment unit admission. To prevent such complications surgeons performing transcervical resection maintain a strict fluid balance (Emanuel et al. 1997). Some surgeons also administer preoperative gonadotrophin hormone releasing hormone (GnRH) analogues in order to reduce operative time and thus the rate of fluid absorption and complications (Donnez et al. 1989). Through their effect on the release on gonadotrophins, GnRH analogues induce a temporary menopause which is thought to reduce fibroid size and improve the operative outcome. However GnRH analogues are associated with considerable side effects including night sweats and hot flushes. Even though

the administration of GnRH analogues before open myomectomy has been shown to reduce blood loss (Lethaby et al. 2001) there is no evidence to support such treatment before transcervical resection of submucous fibroids. The aim of this study is to investigate whether the preoperative administration of GnRH analogues improves the outcome of hysteroscopic resection. Our primary outcome measure was the completeness of resection of the submucous fibroid at TCRF. Secondary outcome measures included: the duration of the TCRF, the fluid deficit recorded at TCRF, the resolution of symptoms postoperatively and the number of subsequent fibroid related operations.

3.2 Population and randomisation

This was a prospective randomized controlled study performed at the EPAGU at KCH. The study was registered on the Current Controlled Trials Website: <http://www.controlled-trials.com/mrct/trial/662485/ISRCTN06560767>.

Patients were recruited from the single stop gynaecological clinic at KCH. The inclusion criteria were: heavy irregular periods and diagnosis of a submucous fibroid on 2D ultrasound. Women who met the inclusion criteria were referred for 3D – SIS by a single experienced operator.

All women with a confirmed Type 1 or Type 2 submucous fibroid on 3D - SIS were given an information leaflet about the study and a written informed consent was obtained from all patients who agreed to take part.

Consecutively numbered, opaque, sealed envelopes were prepared at KCH using a computer – generated simple randomisation sequence. The envelopes were securely kept at the nurses’

office at the EPAGU. Patients were randomly assigned to subcutaneous injections of placebo (5ml of 1% Lignocaine) or Goserelin 3.6mg (Zoladex, AstraZeneca UK, Luton, UK). Randomisation of patients and administration of injections were done by staff nurses who were not part of the trial. Both patients and clinicians involved in the trial were blinded to the group allocation.

A total of three injections were given at four weekly intervals. The operation was scheduled to take place within four weeks from the last injection. Following completion of preoperative treatment patients underwent TCRF. All operations were undertaken by a single expert operator with in excess of 10 years of experience of hysteroscopic surgery and who performs such operations on a weekly basis.

3.3 Statistical analysis

Wamsteker et al. showed that the probability of complete resection of Type O fibroids was 92% per procedure while that of Type 1 and Type 2 fibroids was 60% and 50% per procedure respectively (average 55%) (Wamsteker et al. 1993). Similar figures were also reported by Vercellini et al. and Perino et al. (Perino et al. 1993; Vercellini et al. 1999). Given that it would be desirable to completely remove the fibroid with a single operation our hypothesis was that preoperative use of GnRH analogues would improve the probability of Type 1 and 2 fibroids to be completely resected to that of type O. The study was designed to have an 80% power detect an increase in the proportion of fibroids completely resected at the first operation from 55% in the placebo group to 92% in the treatment group with a two sided α of 0.05. A sample size of 19 was needed in each arm of the study. The analysis was performed on an intention – to – treat basis (Hollis et al. 1999). Multivariate logistic regression analysis

was used to adjust for the influence of age (continuous), fibroid size (continuous), degree of fibroid protrusion (continuous) and group allocation (categorical, 1 = GnRH analogue, 0 = placebo) on the probability of complete fibroid resection (categorical, 1 = complete resection, 0 = incomplete resection).

3.4 Results

The study was performed between the 26th of September 2005 and the 18th of March 2008. 84 women met the inclusion criteria. 37 did not consent to taking part in the trial. As GnRH analogue treatment is not default in our department women refused to participate to avoid the 3 month delay before the operation and the need for monthly injections. Of the 47 women who consented to take part 24 women were randomly assigned to GnRH analogue and 23 to placebo (Figure 17). The demographic and morphologic characteristics of the two groups are presented in Table 11. The groups were balanced in terms of parity, mean diameter of the submucous fibroid, the degree of fibroid protrusion into the endometrial cavity and the proportion of women with multiple fibroids, but women who received GnRH analogues were significantly younger than controls (Table 11). 30/40 (75%, 95% CI 61.6 – 88.4) women who underwent TCRF had complete fibroid resection. In women who had incomplete resections, 8/10 (80%, 95% CI 55.2 – 100.0) procedures were stopped when resection had gone 1cm deep into the myometrium. One procedure was abandoned because of excessive bleeding obscuring the view (1/10, 10% [95% CI 0.0 – 28.6]) and one was abandoned because of a fluid deficit >1.5L (1/10, 10% [95% CI 0.0 – 28.6]). The logistic regression analysis identified fibroid size and degree of fibroid protrusion as independent significant predictors of completeness of fibroid resection (Table 12).

Based on the intention to treat analysis there was no significant difference in the number of complete fibroid resections between women who received GnRH analogues (14/24, 58.3% [95% CI 38.6 – 78.1]) and those that received placebo (16/23, 69.6% [50.8 – 88.4]) (RR 0.84, 95%CI 0.54 to 1.29) ($p= 0.43$). The difference in the proportion of completely resected fibroids between the two groups was 11.3% (95% CI -15.5 – 35.7). A total of 7 women did not undergo planned operation – 3/24 (12.5%, 95% CI 1.4 – 23.6) in the treatment and 4/23 (17.4%, 95% CI 4.4 – 30.4) in the placebo ($p=0.701$). Of the 3 women in the treatment group who did not undergo their operation one developed an allergic reaction, the second one opted for abdominal myomectomy and the third did not attend for her operation. Of the 4 women in the placebo group who did not undergo their operation, one opted for abdominal myomectomy and the remaining three did not attend for their operation.

The results in the subgroup of women who underwent surgery are shown in Table 13. Again there was no significant difference in the number of complete resections between women in the study compared to the placebo group (14/21, 66.7% [95% CI 46.5 – 86.8] vs. 16/19, 84.2% [95% CI 67.8 – 100.0] respectively; RR 0.79 [0.55 – 1.13] $p=0.20$). The difference in the proportion of completely resected fibroids between the two groups was 17.5% (95% CI -12.9 – 43.8). The mean volume of fluid infused during hysteroscopy (7.7L [\pm 4.9] vs. 9.3 [\pm 5.7] respectively; $p=0.39$), the number of women who had fluid deficit in excess of 1.5L (12.5% [95% CI 0.0 – 25.7] vs. 4.3% [95% CI 0.0 – 12.7] respectively; RR 2.71 [0.31 – 23.93] $p=0.38$), the mean duration of the procedure (30.0 min [20.0 – 40.0] vs. 30.0 min [21.0 – 31.0] respectively; $p=0.84$) and complication rate (4.2% [95% CI 0.0 – 12.2] vs. 8.7% [95% CI 0.0 – 20.2] respectively; RR 0.45 [0.04 – 4.6] $p=0.56$) were also similar between the two groups (Table 13).

One woman who received GnRH analogue suffered a uterine perforation and bowel injury that necessitated laparotomy and repair. She eventually made a full recovery. Two women in placebo group suffered excessive intraoperative bleeding, which was controlled by the insertion of a Foley catheter in one and cervical suture in the other case.

36/40 (90%) of women who were operated attended for a six week follow up visit. 15/20 women that were allocated to GnRH analogues felt that their symptoms were had resolved compared to 7/16 women that were allocated to placebo (75.0% [95% CI 56.0 – 93.9] vs. 43.8% [95% CI 19.4 – 68.1] respectively; RR 1.7 [0.93 – 3.2] p=0.084).

Until the 3rd of October 2009, 10 women had undergone a second operation for their fibroids. 4/20 women that received GnRH analogues compared to 6/16 that received placebo had a second operation ([20.0%, 95% CI 2.5 – 37.5] vs. [37.5%, 13.8 – 61.2] respectively; RR 0.53 [0.18 – 1.57], p=0.25). 8/10 (80%, 95% CI 55.2 – 100.0) women had a repeat intervention because their symptoms of menorrhagia did not resolve. One woman was operated because of several non - submucous fibroids (1/10, 10% [95% CI 0.0 – 28.6]) and one developed a new submucous fibroid (1/10, 10% [95% CI 0.0 – 28.6]).

Figure 17 Randomised controlled trial for the preoperative use of GnRH analogues in women with submucous fibroids scheduled for TCRF. Flow of participants through the randomisation process (n=84)

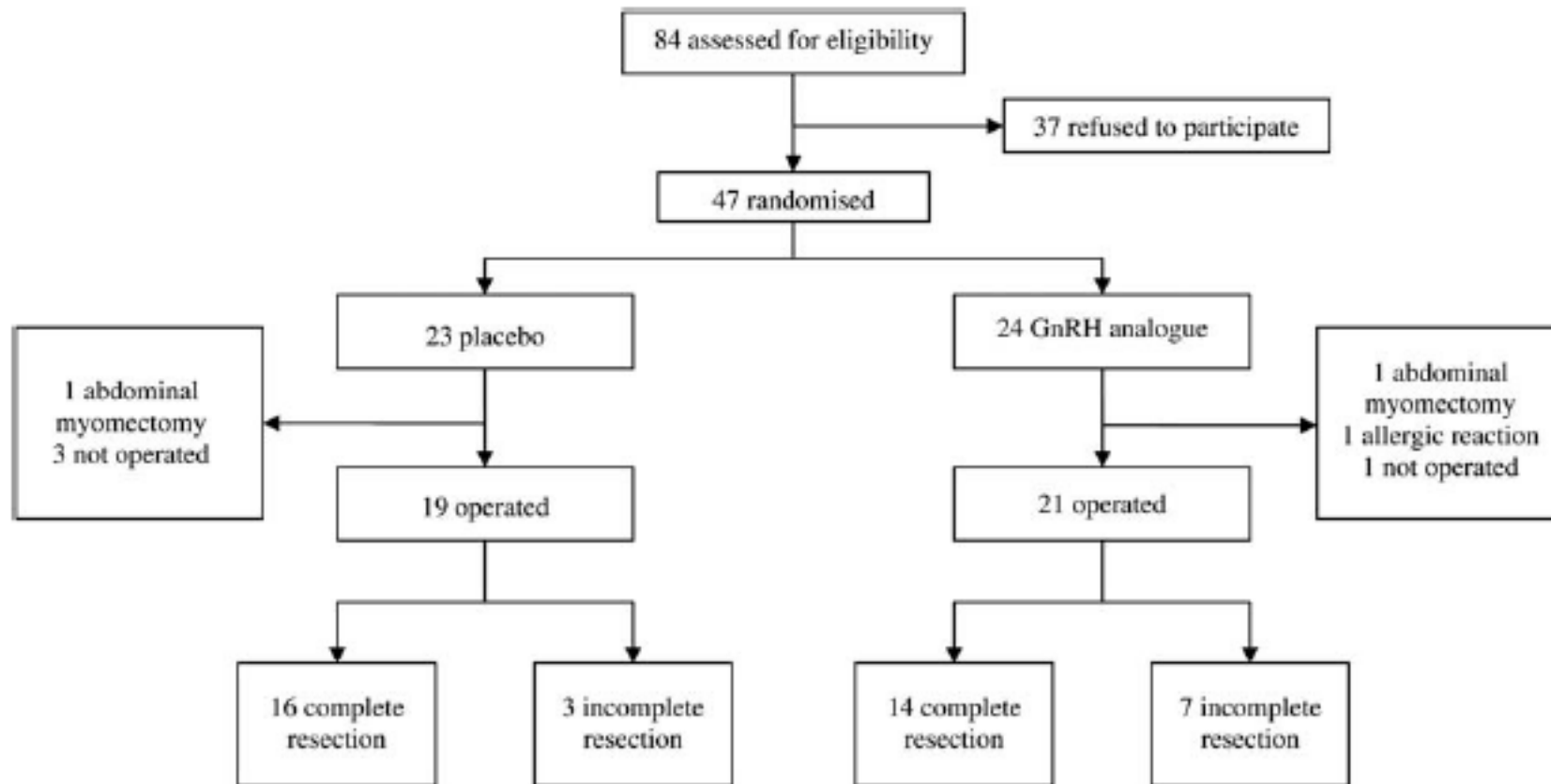


Table 11 Comparison of baseline characteristics of patients participating in the randomised controlled study and randomised to GnRH (n=24) and placebo (n=23), intention – to – treat analysis

	GnRH (n=24)	Placebo (n=23)	P
Mean age (SD), years	38.8 (7.6)	44.5 (5.5)	0.005
Median parity, (range)	1 (0-3)	2 (0-3)	0.327
Median fibroid diameter, mm (IQR)	29.0 (24.5 – 40.25)	29.0 (21.25 – 35.5)	0.237
Mean degree of protrusion, % (\pm SD)	71 (19)	68 (21)	0.820
Multiple fibroids, n (% , 95% CI)	4 (16.7, 1.8 – 31.6)	4 (17.4, 1.9 – 32.9)	1.0

Table 12 Multivariate logistic regression to predict completeness of resection (dependent) based on age, parity, treatment with GnRH analogue, fibroid diameter and the degree of fibroid protrusion (independent variables)

	OR (95% CI)	P
Age	1.015 (0.886 – 1.164)	0.828
Parity	0.938 (0.371 – 2.371)	0.892
GnRH	1.471 (0.184 – 11.760)	0.716
Fibroid diameter, mm	0.921 (0.857 – 0.990)	0.025
Degree of protrusion, % (\pm SD)	1.086 (1.002 – 1.176)	0.044

Table 13 Comparison in primary and secondary outcomes between women who underwent TCRF and were randomised either to GnRH (n=21) or placebo (n=19)

	GnRH (n=21)	Placebo (n=19)	RR (95%CI)	P
Intention to treat complete resection (%,95% CI)	14/24 (58.3, 38.6 – 78.1)	16/23 (69.6, 50.8 – 88.4)	0.84 (0.54 – 1.29)	0.43
Complete resection n, (% , 95% CI)	14 (66.7, 46.5 – 86.8)	16 (84.2, 67.8 – 100.0)	0.79 (0.55–1.13)	0.20
Mean volume of fluid infused L, (±SD)	7.7 (4.9)	9.3 (5.7)	-	0.39
Fluid deficit >1500ml n, (% , 95% CI)	3 (12.5, 0 – 25.7)	1 (4.3, 0 – 12.7)	2.71 (0.31- 23.93)	0.38
Median volume of fluid deficit L, (range)	0.30 (0.0 – 1.3)	0.50 (0.0 – 0.975)	-	0.84
Median duration of operation min, (range)	30.0 (20.0 – 40.0)	30.0 (21.0 – 31.0)	-	0.84
Complications n, (% , 95% CI)	1 (4.2, 0 – 12.2)	2 (8.7, 0 – 20.2)	0.45 (0.04-4.6)	0.56

[4] Three dimensional power Doppler in the diagnosis of endometrial cancer

4.1 Background

Women with endometrial cancer usually present with a change in bleeding patterns or more commonly bleeding after the menopause (Lidor et al. 1986) . Because most women develop symptoms and present early to medical practitioners most endometrial cancers are diagnosed at an early stage with 5 – year survival over 85% (Creasman et al. 2006). In a population of women with postmenopausal bleeding the prevalence of endometrial cancer is up to 15% (O'Connell et al. 1998). For women with suspected endometrial cancer the initial method of assessment is transvaginal ultrasound in order to measure the thickness of the endometrium (Gupta et al. 2002). Women found to have endometrial thickness less than 5mm have a significantly reduced risk of having endometrial cancer and so these patients can be safely discharged. In contrast, the positive predictive value of the test is relatively low and so patients who test positive need some form of endometrial sampling to confirm the diagnosis. Because of the poor positive predictive value of the test the majority of these patients will have a negative finding and will be discharged. In addition to the inconvenience to the patient and the risk of complications, endometrial sampling poses a significant burden on healthcare systems as it often requires day case admission and general anaesthesia. Attempts have been made to improve the performance transvaginal ultrasound for screening women with suspected endometrial cancer using three dimensional volume measurement of the

endometrium (Gruboeck et al. 1996) and uterine artery Doppler (Bourne et al. 1991) but these have not been particularly successful. Recently, as an adjunct to endometrial volume measurement, power Doppler has been used to objectively quantify the vascularity of the endometrium (Pairleitner et al. 1999). Some have used this method as a screening tool in women with postmenopausal bleeding and a thick endometrium with encouraging results (Odeh et al. 2007; Alcazar et al. 2009) but these studies suffer various methodological limitations. The aim of our study was to evaluate 3D - PDA as a second line test for the detection of endometrial cancer in women with postmenopausal bleeding and an endometrial thickness over 5mm.

4.2 Methods

This was a prospective study over a period of 24 months. Women with post menopausal bleeding were referred by their general practitioners or gynaecologists to our dedicated post menopausal bleeding clinic for assessment. Menopause was defined as amenorrhea for 1 year or more after the age of 45. All women underwent 2D ultrasound scan and measurement of the endometrial thickness. Those found to have endometrial thickness more than 5mm underwent 3D – PDA as described in the methods section. We included all women with postmenopausal bleeding and endometrial thickness greater than 5mm for whom 3D – PDA volumes were available for analysis. We excluded women who were unable to undergo transvaginal ultrasound (virgo intacta/ unable to consent), those who were taking hormone replacement therapy and if the ultrasound examination revealed fluid in the endometrial cavity. Following the ultrasound examinations patients underwent endometrial sampling. Women with a focal lesion were scheduled for hysteroscopy and endometrial biopsy. Women with a global lesion underwent Pipelle endometrial biopsy. If the Pipelle examination was

reported as inadequate the women were scheduled for hysteroscopy. None of the patients had endometrial sampling prior to ultrasound assessment. After the examination endometrial volume and the vascular indices were calculated as described in the methods section.

4.3 Statistical analysis

The Kolmogorov - Smirnov test rejected the normal distribution for endometrial thickness, endometrial volume, VFI, VI and FI. Non - normally distributed variables as median (IQR) and normally distributed variables as mean (SD). The data was split into sets consisting of a training and a testing set for the multivariate logistic regression analysis. The statistical package was programmed to randomly select 65% of all cases. This process produced the training set on which the logistic regression was carried out. The remainder of the cases were used as a testing set on which the derived regression equation was cross – validated. The randomly selected training set contained 34 patients with 9 cancers and 25 benign cases. The testing set contained 18 cases with 8 cancers and 10 benign cases. Forward conditional stepwise multivariate logistic regression was used to identify independent predictors of malignancy (categorical, benign vs. malignant). The initial model included age (continuous), endometrial thickness (continuous), endometrial volume (continuous), VI (continuous), VFI (continuous) and FI (continuous). We plotted ROC curves for the predicted probability for the training and testing sets and calculated the AUC.

4.4 Results

During the study period of 28 months 91 women had an endometrial thickness of >5mm. 22/91 (24.2%, 95% CI 15.4 – 33.0) were diagnosed with endometrial cancer, 4/91 (4.4%, 95% CI 0.2 – 8.6) with simple hyperplasia, 1/91 (1.1%, 95% CI 0.0 – 3.2) with hyperplasia

with atypia, and the remaining 64/91 (70.3%, 95% 60.9 – 79.7) with a variety of benign diagnoses. In 52/91 (57.1%, 95% CI 47.0 – 67.3) women endometrial volume was available for analysis (Table 14).

The median endometrial thickness was 10.4 mm (IQR, 8.0 – 17.3 mm). The median endometrial volume was 4.2 cm³ (IQR, 2.3 – 10.6). The median VI, VFI and FI were 1.6 (IQR, 0.3 – 5.3), 0.43 (IQR, 0.07 – 1.78) and 29.1 (IQR, 23.2 – 33.8) respectively. The performance of individual ultrasonic variables to discriminate between benign and malignant endometria is shown in (Table 15).

There was a significant difference in age ($p=0.037$), endometrial thickness ($p=0.001$), endometrial volume ($p=0.003$), VI ($p=0.018$), VFI ($p=0.020$), and FI ($p=0.008$) between women with cancer and those with benign pathology. The diagnostic performance of individual ultrasonic variables is show in Table 16. Multivariate logistic regression for the training set ($n=34$) selected endometrial thickness and VFI as best independent predictors of malignancy or benignity. In the training set the model had an AUC of 0.833 (SE 0.082) with 55.6% (95% CI 23.1 – 88.0) sensitivity and 95.8% (95% CI 87.8 – 100) specificity. When this model was applied to testing set ($n=28$) the AUC was 0.875 (SE 0.117) with 75% (95% CI 45.0 - 100) sensitivity and 100% specificity ($p>0.05$).

Table 14 List of histological diagnoses in women with endometrial thickness >5mm who underwent endometrial sampling (n=91)

Histological diagnosis	n (%)
Benign endometrial polyp	19 (36.5)
Proliferative endometrium	7 (13.5)
Atrophic endometrium	6 (11.5)
Simple endometrial hyperplasia	2 (3.8)
Fibroid	1 (1.9)
Endometrial endometrioid adenocarcinoma	9 (17.3)
Complex hyperplasia with atypia	3 (5.8)
Serous carcinoma	2 (3.8)
Squamous cell carcinoma	2 (3.8)
Carcinosarcoma	1 (1.9)
Total	91 (100)

Table 15 Comparison of demographic and ultrasonic variables in women with postmenopausal bleeding and endometrial thickness > 5mm diagnosed benign pathology (n= 35) and endometrial malignancy (n=17)

	Endometrial cancer (n=17)		Benign (n=35)		
	Median	IQR	Median	IQR	Sig.
Age (years)	64	59 – 73.5	59	54 - 66	0.037
Endometrial thickness (mm)	17.7	10.4 – 25.7	8.8	7.4 – 14.0	0.001
Endometrial volume (cm ³)	9.8	3.6 – 18.4	3.8	1.8 – 6.2	0.003
VI	3.1	0.9 – 11.9	0.7	0.1 – 3.5	0.018
VFI	1.1	0.2 – 4.6	0.2	0.02 – 1.0	0.020
FI	33.2	26.3 – 42.4	25.9	21.3 – 32.5	0.008

Table 16 Diagnostic performance of individual ultrasonic variables for the discrimination between benign and malignant endometria in women with postmenopausal bleeding and endometrial thickness >5mm (n=52)

Variable	Cut off	Sensitivity	Specificity	AUC (95% CI)
Endometrial thickness (mm)	10.1	82.4	68.6	0.825 (0.698 – 0.953)
Endometrial volume (ml)	5.2	64.7	71.4	0.753 (0.610 – 0.896)
VI	1.4	76.5	61.3	0.708 (0.551 - 0.864)
VFI	0.586	70.6	67.7	0.705 (0.545 - 0.854)
FI	30.2	64.7	64.5	0.734 (0.585 - 0.864)

[5] Three dimensional ultrasound for the prediction of endometrial cancer stage

5.1 Background

Based on current FIGO guidelines all women with a diagnosis of endometrial cancer should undergo surgical staging of the disease with lymph node sampling or lymphadenectomy (Creasman et al. 2006). It has been suggested that routine lymphadenectomy may have therapeutic benefits (Chan et al. 2006) but this has not been confirmed in recent studies (Kitchener et al. 2009). In addition, several large studies have demonstrated that adjuvant radiotherapy may not be beneficial in women with early disease (Creutzberg et al. 2000; Keys et al. 2004; Johnson et al. 2007; Blake et al. 2009). These developments have lead to increasing recognition that treatment of endometrial cancer should be individually tailored, taking into account the woman's risk of having extrauterine disease. This can only be achieved if reliable, preoperative, information about the extent of the disease is available to attending clinicians. Studies on histopathological specimens have demonstrated that the probability of lymph node involvement correlates with tumour size (Schink et al. 1987; Mariani et al. 2000) and the degree of cellular differentiation (Creutzberg et al. 2001). Attempts to predict the extent of endometrial cancer from 2D ultrasound scan findings have

been made previously with mixed results (Cagnazzo et al. 1992; Cheng et al. 1998; Sawicki et al. 2003; Eitan et al. 2005; Savelli et al. 2008). Introduction of 3D ultrasound systems has allowed for accurate measurements of endometrial volume to be performed preoperatively (Raine-Fenning et al. 2003; Raine-Fenning et al. 2003). Several studies have suggested that endometrial volume may be superior to endometrial thickness for the differential diagnosis between benign and malignant endometrial lesions in symptomatic postmenopausal women (Gruboeck et al. 1996; Mansour et al. 2007). There is also some limited evidence suggesting that endometrial volume is higher in women with poorly differentiated tumours and in those with more advanced disease. The aim of this study was to investigate whether endometrial volume as measured by 3D ultrasound, alone or in combination with other variables, could be used preoperatively to predict the stage of endometrial cancer.

5.2 Methods

This was a prospective study of women with a history of post menopausal bleeding who were referred to our dedicated rapid access post menopausal bleeding at the EPAGU and GDTU for assessment and ultrasound scan. Women using hormone replacement therapy were not included in the study. None of the patients had dilatation and curettage or endometrial biopsy prior to assessment. All women were seen by gynaecologists who were highly skilled in transvaginal ultrasound and offered an ultrasound examination to assess the endometrium. Once the measurement of endometrial thickness was completed if endometrial thickness was $\geq 5\text{mm}$, three – dimensional volumes were acquired and stored for later analysis as described in the methods section. Endometrial sampling was then performed as appropriate. Women diagnosed with endometrial cancer were then referred to the onco – gynaecologist for

treatment. Once women had undergone definitive treatment the results of surgical staging were recorded and stored in a purpose built database.

5.3 Statistical analysis

For the purposes of this study we defined “need for adjuvant therapy” as stage of endometrial cancer \geq II. We plotted ROC curves assess the ability of individual preoperative variables to predict the stage of endometrial cancer. All the variables (age, endometrial thickness, endometrial volume, histological grade) were then entered into forward conditional logistic regression to select the best independent predictors of the stage of endometrial cancer (Stage \geq II = 1, Stage < II = 0). Variables were retained in the model with a $p < 0.05$. The Hosmer – Lemeshow test was used to assess goodness – of – fit for the model.

5.4 Results

173 women with a median age of 62.0 years (IQR, 55.0 – 72.0) were included in the study. 134/173 (77.5% 95% CI 71.2 – 83.7) had benign pathology and 39/173 (22.5% 95% CI 16.3 – 28.8) were diagnosed with endometrial cancer. (Table 17) Women with benign pathology were significantly younger and had a significantly smaller median endometrial thickness and median volume compared to those diagnosed with endometrial cancer. (Table 18)

In women with endometrial cancer, endometrial sampling revealed Grade I tumour in 10/39 (25.6%, 95% CI 11.9 – 39.3) women, Grade II in 10/39 (25.6%, 95% CI 11.9 – 39.3) and Grade III in 19/39 (48.7%, 95% CI 33.0 – 64.4). 5/39 (12.8%, 95% CI 2.3 – 23.3) women were not operated on because of co – morbidities precluding safe conduct of surgery.

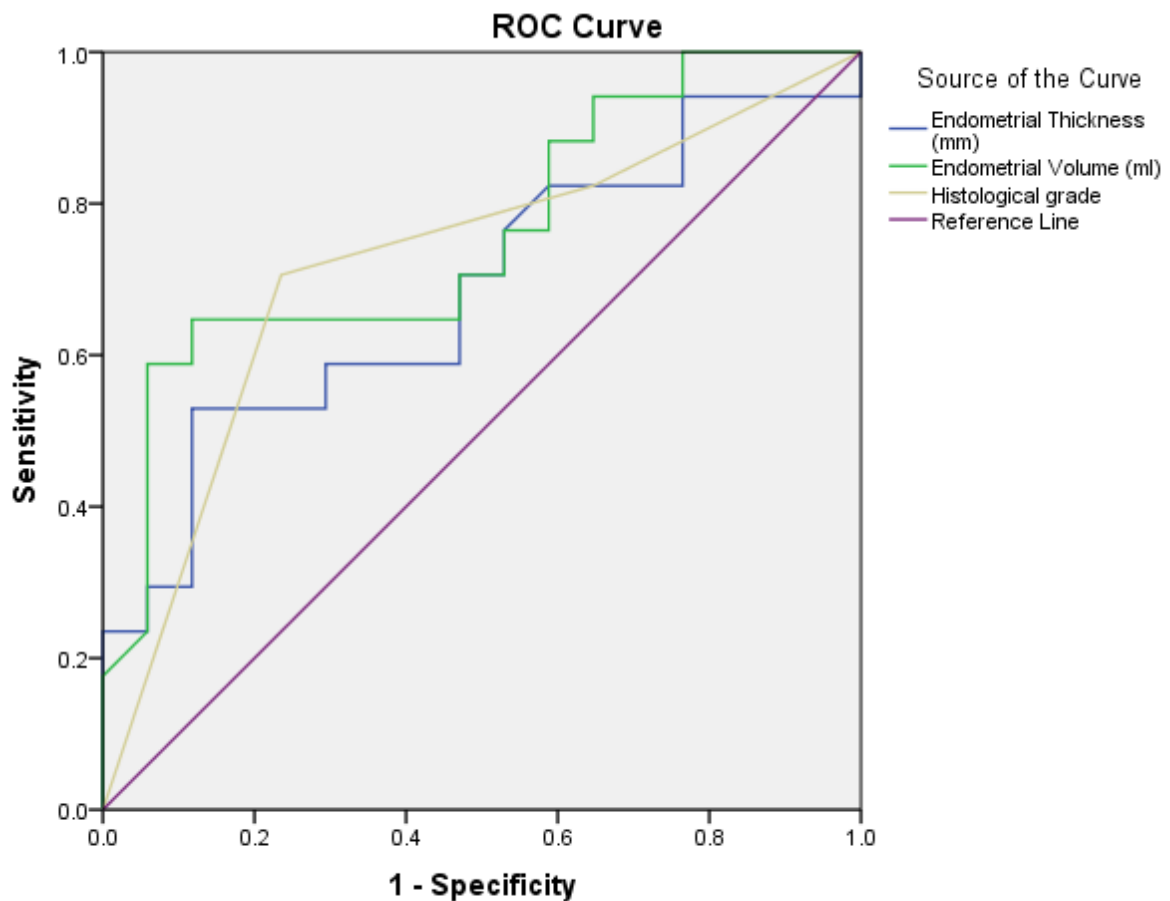
Histopathology examination of the hysterectomy specimen revealed Grade I disease in 7/34 (20.6%, 95% CI 7.0 – 34.2) women, Grade II disease in 10/34 (29.4%, 95% CI 14.1 – 44.7) and Grade III disease in 17/34 (50.0%, 95% CI 33.2 – 66.8). In 4/34 (11.8%, 95% CI 0.9 – 22.6) cases the final histological grade of the tumor based on the examination of hysterectomy specimen was different from the histological grade estimated at endometrial sampling. In two cases the initial Grade I histology was changed to Grade II, in one case the initial Grade II histology was changed to Grade III. In one case the Pipelle biopsy revealed Grade II pathology however the predominant pattern in the resection specimen was Grade I. The various histological subtypes are shown in Table 19 .

Of the 34 women who underwent operative staging at laparotomy, 17/34 (50.0%, 95% CI 33.2 – 66.8) were staged as Stage I, 6/34 (17.7%, 95% CI 4.8 – 30.5) as Stage II, 8/34 (23.5%, 95% CI 9.3 – 37.8) as Stage III and 3/34 (8.8%, 95% CI 0.0 – 18.4) as Stage IV. 17/34 women (50.0%, 95% CI % 23.8 – 76.2) required adjuvant radiotherapy according to the management criteria outlined in our methods.

There was a significant positive correlation between endometrial cancer grade on endometrial sampling and disease stage ($r = 0.354$, $p = 0.027$) as well as between endometrial volume and disease stage ($r = 0.385$, $p = 0.015$). There was no significant correlation between endometrial thickness and disease stage ($p = 0.227$). Stepwise forward conditional logistic regression retained histological grade on endometrial sampling ($p = 0.058$, OR 2.77 [95% CI 0.97 – 7.96]) and endometrial volume ($p = 0.041$, OR 1.040 [1.002 – 1.080]) as the best independent

predictors of the extent endometrial cancer spread age ($p=0.324$) and endometrial thickness ($p=0.214$) were not retained in the model. The receiver operating characteristic curves for individual variables and for the logistic model is shown in Figure 19 and Table 20.

Figure 18 ROC curves for individual variables for the prediction of endometrial cancer stage (n=34). Endometrial thickness (AUC = 0.683), endometrial volume (AUC=0.760), histological grade on endometrial sampling (AUC= 0.720)



Diagonal segments are produced by ties.

Figure 19 ROC curve for the logistic regression model including endometrial volume and histological grade on endometrial sampling (independent variables) for the prediction of endometrial cancer II or higher (dependent variable) (n=34)

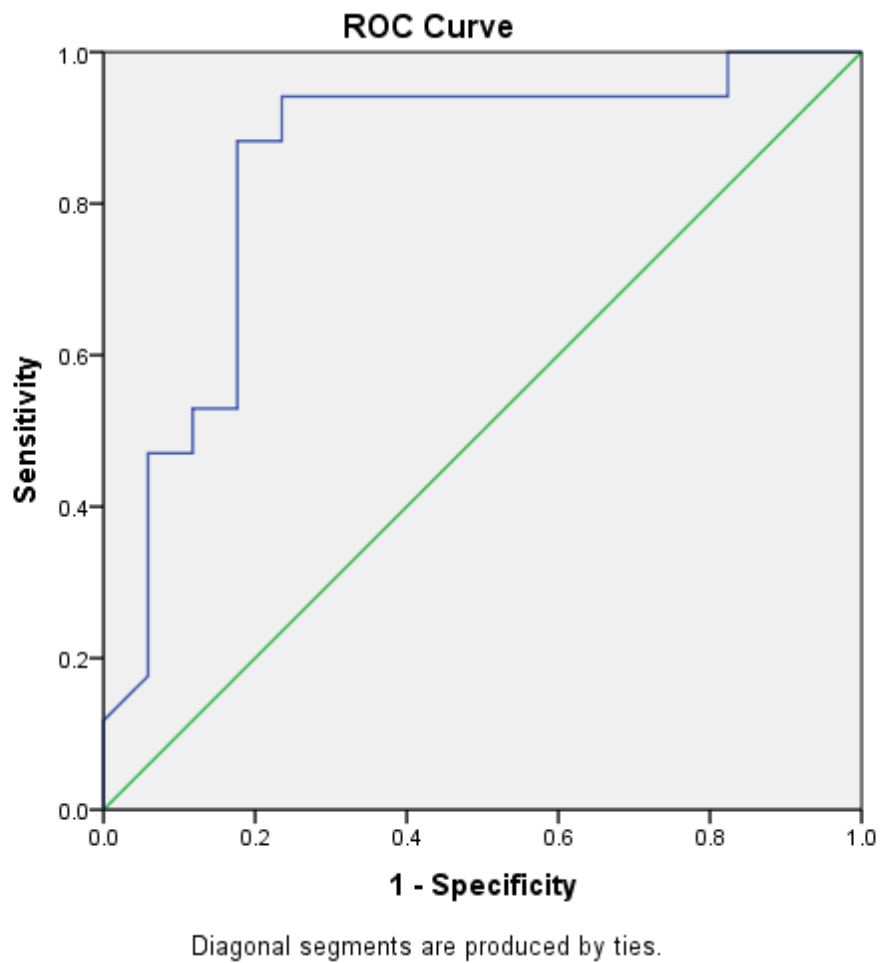


Table 17 List of histological subtypes in women with postmenopausal bleeding and endometrial thickness >5mm (n=173)

Histological diagnosis	n (%)	
Benign endometrial polyp	42	(24.3)
Atrophic endometrium	33	(19.1)
Proliferative endometrium	30	(17.3)
Endometrial hyperplasia	22	(12.7)
Other benign endometrial pathology	7	(4.1)
Endometrial cancer	39	(22.5)
Total	173	(100)

Table 18 Comparison of age, endometrial thickness and volume in postmenopausal women diagnosed with benign (n=134) and malignant (n=39) pathology

	Benign (n=134)	Cancer (n=39)	p
Median age (years) (25 th – 75 th centile)	57.0 (54.0 – 67.0)	66.0 (59.0 – 77.0)	0.012
Median endometrial thickness (mm) (25 th – 75 th centile)	8.8 (7.1 – 14.0)	25 (12.4 – 31.3)	<0.001
Median endometrial volume (mls) (25 th – 75 th centile)	4.1 (2.3 – 6.4)	21.3 (8.5 – 40.6)	<0.001

Table 19 Distribution of endometrial cancer histological subtypes in women with postmenopausal bleeding and endometrial thickness $\geq 5\text{mm}$ (n=34)

Histological diagnosis	n (%)
Adenocarcinoma	28(82.4)
Papillary serous	3(8.8)
Adenosquamous	2(5.9)
Mixed clear cell and adenocarcinoma	1(2.9)
Total	34(100)

Table 20 Summary of the performance of individual variables and a logistic regression model including endometrial volume and histological grade at endometrial sampling to predict endometrial cancer stage II or higher (n=34)

	Cut off	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	+ve LR (95% CI)	-ve LR (95% CI)	AUC (95% CI)
Endometrial thickness (mm)	16.9mm	12/17 (70.6 [44.0 – 89.7])	9/17 (52.9 [27.8 – 77.0])	1.5 (0.8 – 2.7)	0.56 (0.2 – 1.3)	0.683 (0.501 – 0.866)
Endometrial volume (ml)	14.7ml	12/17 (70.6 [44.0 – 89.7])	9/17 (52.9 [27.8 – 77.0])	1.5 (0.8 – 2.7)	0.56 (0.2 – 1.3)	0.760 (0.595 – 0.924)
Histological grade on endometrial sampling	2	14/17 (82.4 [56.6 – 96.2])	6/17 (35.3 [14.2 – 61.7])	1.27 (0.8 – 1.9)	0.50 (0.2 – 1.7)	0.720 (0.541 – 0.898)
Endometrial volume + Histological grade	44%	15/17 (88.2 [63.6 – 98.5])	14/17 (82.4 [56.6 – 96.2])	5.0 (1.8 – 14.2)	0.14 (0.04 – 0.5)	0.849 (0.708 – 0.991)

PART IV Discussions

[1] Natural history of fibroids

Our study showed that in premenopausal women with uterine fibroids, the volume of the largest fibroid increases by a median 35% per year. We found that the growth rate of fibroids shows considerable variability and, in contrast to traditional teaching, that up to one fifth of fibroids in premenopausal women may spontaneously regress. Despite the widespread prevalence of fibroids there are only 2 studies in the literature on the natural history of fibroids, which makes patient counselling and management difficult. Recently Peddada et al. in a study of 262 fibroids in 72 women examined using MRI reported that the median growth rate for fibroids was 9% in six months and that the growth rate of individual fibroids was slower in women with multiple tumours (Peddada et al. 2008). They also found that 7% of the fibroids in the study decreased in volume by more than 20% in 6 months and described these fibroids as ‘regressing’. In our dataset only 3% of the fibroids showed similar behaviour. However, in our study approximately 40% of the participants had a single tumour in contrast to Peddada et al. in which more than 90% of participants had multiple fibroids. This difference in population and the fact that we restricted our analysis to the largest fibroid in each woman may account for the differences in growth rate and proportion of regressing fibroids between studies. We found only one other ultrasound study of the natural history of fibroids in the literature. It was performed by DeWaay et al. in 2002 and reported an average 1.2cm increase in diameter per 2.5 years. However this study was based on a very small

sample, 18 fibroids, and the change in mean fibroid diameter over the examination interval did not reach statistical significance (DeWaay et al. 2002; Peddada et al. 2008).

Our study found a surprising degree of heterogeneity in fibroid growth rate. This finding is similar to the findings by Peddada et al. and so it is supported by the current literature (Peddada et al. 2008). Nevertheless we are conscious of the possibility that a potential source of this variability may have been measurement error rather than true change in fibroid diameter. In order to minimise this source of variability we restricted our analysis to a single expert operator and the largest fibroid which should have reduced operator bias. The validity of transvaginal ultrasound for fibroid measurement has been shown before in a study where the gold standard was histological assessment (Dueholm et al. 2002) which is reassuring for the accuracy of our results.

Our results suggest that fibroid size at the initial scan can be used to assess its potential to grow in the future. We found that fibroids measuring less than 20 mm and over 50 mm diameter at presentation have a growth rate up to three times higher in comparison to fibroids measuring between 20 and 50mm diameter. This is in contrast to the findings of Peddada et al. (Peddada et al. 2008) who did not detect a significant difference in the growth rate between different size fibroids. A limitation of their study, however, was that they only included women with at least one fibroid over 50mm and that they could not measure accurately fibroids less than 15mm in diameter. This may have led them to underestimate the effect of size on growth rate. Our results suggest that fibroids do not continue growing at a constant rate indefinitely, i.e. that fibroid growth is not linear despite a stable hormonal environment. It may be the case that fibroids start by growing at a fast pace and then growth slows once the tumour reaches a certain size. The maximum size might be determined by

non-hormonal factors such as availability of blood supply to support fibroid growth. Interestingly, recent cytogenetic studies have demonstrated an association between fibroid mean diameter and fibroid karyotype (Flake et al. 2003). Rein et al. (Rein et al. 1998) and Hennig et al. (Hennig et al. 1999) demonstrated, in histopathological specimens, that fibroids with a chromosome 12;14 translocation had significantly larger mean diameters (8 - 9 cm) compared to fibroids with chromosome 7 deletions (3 - 5 cm). Fibroids with a mosaic pattern were of intermediate diameter compared to abnormal non-mosaic fibroids whilst fibroids with apparently normal karyotype were the smallest in both studies.

In addition to fibroid initial size, we found that fibroids in different locations have significantly different growth rates. Intramural fibroids demonstrated the fastest growth followed by subserous and submucous fibroids. However, the myometrium is relatively thin and fibroids over 30–40 mm are inevitably classified as subserous or submucous. Indeed in our study intramural fibroids were significantly smaller compared to subserous and submucous fibroids. The results of our multivariate analysis confirm that whilst fibroid size independently influences growth rate, the association between position and growth rate is a reflection of the effect of size on growth rate rather than an independent effect of fibroid position.

Women with fibroid-related symptoms (menorrhagia, clinically palpable tumour) had significantly higher median fibroid volume at presentation compared to women who presented for other reasons. Others have reported that fibroid size plays a pivotal role in fibroid symptomatology (Wegienka et al. 2003) and our results are in agreement with this. The prevalence of fibroids increases with age (Marshall et al. 1997) and some have speculated that this increase in prevalence is due to accelerated fibroid growth in later life

secondary to hormonal changes. Our results did not confirm this theory and if anything suggest that women over 35 tend to have slower fibroid growth rates although this relationship did not reach statistical significance. A similar decline in fibroid growth rates with age in Caucasian women was recently reported by Peddada et al. (Peddada et al. 2008), who used MRI for their measurements. It is possible that the reported increased prevalence of fibroids in later life is due to a prolonged period of sustained growth rather than a tendency of fibroids to grow faster in older women.

The retrospective nature of our study precluded us from identifying specific factors which may have affected fibroid development. A future study to look at fibroid development prospectively should record fibroid size at regular intervals while controlling for variables which may have affected fibroid volume such as such as time in the menstrual cycle, body mass index and racial origin which may explain part of the variability we recorded here.

The heterogeneity in fibroid growth rate makes it difficult to make firm predictions about behaviour of individual fibroids. Indeed, a minority of fibroids in our study demonstrated evidence of spontaneous regression, which is an unexpected finding in premenopausal women. Until now fibroid regression had been assumed to occur only after the menopause secondary to the reduction in circulating ovarian steroids. DeWaay et al. (DeWaay et al. 2002) suggested that this regression is secondary to women being in the peri-menopause, but our study included only women with regular menses and so this is an unlikely explanation. We also excluded any women on exogenous hormonal treatment and so we can speculate that individual fibroids may have regressed secondary to local effects, such as a change in vascularity. Clearly further investigation into the characteristics of regressing fibroids is necessary.

We conclude that fibroid growth is highly variable and women's age as well as tumour size at presentation can give indication as to the likely course of the tumour's future development. Importantly, fibroids can regress spontaneously, even in premenopausal women, and therefore interventions should be planned only after a period of observation to establish the tumour behaviour in individual cases.

[2] Three dimensional saline infusion sonohysterography for the preoperative prediction of fibroid resectability

Our study showed that the outcome of hysteroscopic resection of submucous fibroid is significantly influenced by parity, the overall size of the submucous fibroid and the size of its intramural component. This study is the first to correlate demographic and ultrasonic findings to surgical outcome and thus gives valuable information for the preoperative counselling of patients. Our results confirm the initial observation by Wamsteker et al. (Wamsteker et al. 1993), who suggested that the degree of protrusion of submucous fibroid into the uterine cavity can be used to assess the success of hysteroscopic resection of fibroid. Wamsteker's classification does not include the size of submucous fibroid, which is a possible weakness as most surgeons would agree that the likelihood of successful fibroid resection tends to decrease with increasing fibroid size. We hypothesise that the omission of the fibroid size from the current classification could be explained by limitations of diagnostic hysteroscopy which does not allow for the accurate measurement of fibroid size. Indeed Cicinelli et al. found that there was significant difference between fibroid diameter estimation at hysteroscopy and the measurement at histopathology (Cicinelli et al. 1995). Previous studies have also alluded to the importance of fibroid size in determining the outcome of resection. Vercellini et al. suggested that fibroids measuring >30 mm in diameter should only be operated on by expert surgeons if a complete resection is to be accomplished in a single

procedure (Vercellini et al. 1999). This recommendation, however, is not based on scientific data but it appears to be based on the author's personal experience. Although Emanuel et al. did not assess fibroid size in their analysis of fluid deficit at TCRF (Emanuel et al. 1997) they did suggest that the size may be an important determinant of the amount of fluid loss. Hart et al. found that the fibroids over 30mm in diameter are more likely to require two stage procedures (Hart et al. 1999). In our study the risk of incomplete resection was significantly higher in fibroids > 38 mm is size, which is in agreement with the previously mentioned studies.

Our results show that the size of the intramural component of the fibroid is also important. This may be because in smaller fibroids resection can be successful even if the protrusion ratio is small. With increasing size of the intramural component the procedure becomes more difficult irrespective of the protrusion ratio. This may explain why in our multivariate analysis the size of intramural component is more significant than the protrusion ratio in predicting complete resection.

To assess the relative importance of the various significant predictors of complete resection and to create a clinically useful tool we performed multivariate analysis. This resulted in a logistic regression equation that can be used preoperatively to estimate the probability of fibroid resection for individual women. Apart from improving patient counselling, preoperative knowledge of a low probability of a successful single stage operation would reduce the pressure on the operating surgeon to remove all of the fibroid in a single session which may in turn lead to a reduction in complications.

An attempt at a novel submucous fibroid classification system has been previously made before by Lasmar et al. (Lasmar et al. 2005) who proposed a complex classification based on a number of parameters (penetration of the nodule into the myometrium, extension of the base of the nodule with respect to the wall of the uterus, size and topography) determined using a variety of diagnostic methods (transvaginal ultrasound, magnetic resonance imaging and hysteroscopy). The system may not be easy to apply in clinical practice and reproducibility and accuracy has not been assessed. Our model is much simpler to use and can generate the probability score for individual women.

Previous investigators in our unit have shown good agreement between 3D – SIS and diagnostic hysteroscopy to classify submucous fibroids based on the degree of protrusion into the endometrial cavity (Salim et al. 2005). To date 3D – SIS remains the only method for which the reproducibility of measurements for the classification of submucous fibroids has been shown to be good (Lee et al. 2006). The results of this study demonstrated that 3D – SIS offers additional information above what is available at hysteroscopy that are useful for the pre - operative planning. These findings suggest that 3D – SIS is the ideal method for the investigation of submucous fibroids and may be the gold standard.

While our methodological design eliminates the problem of inter – observer variability by using a single operator for ultrasound and operative procedures, it does represent conditions which are difficult to reproduce in routine clinical practice. In this study we used intraoperative estimation of complete fibroid resection as our endpoint. We chose this endpoint because it allows direct comparison with the initial study in this field by Wamsteker et al. (Wamsteker et al. 1993). However this endpoint is vulnerable to operator bias which may have been exacerbated by the fact that it was not possible to blind our surgeon to the

ultrasound result. In order to mitigate this risk we used strict criteria to define completeness of resection which should improve the reliability of our results. A future study investigating the performance of 3D – SIS should use more patient centred outcomes such as menstrual calendars and postoperative satisfaction which provides more valuable information. In contrast complete vs. incomplete resection does not necessarily reflect resolution of the patient's symptoms. To avoid the subjective nature of our primary endpoint future studies should use post operative 3D – SIS as an alternative. This is more appropriate as it is more valid and reproducible. Our single centre setup inevitably resulted in a relatively small sample size and it is therefore essential that our classification is subjected to prospective audit in individual units before it could be used in patient selection and counselling.

[3] Randomised controlled trial for the use of GnRH before transcervical resection of fibroid

Our study did not demonstrate a benefit of preoperative administration of GnRH in increasing the proportion of complete resections of Type I and II fibroids at TCRF. However, the overall success rate of TCRF was 75%, which was higher than assumed in our power calculation. This could be explained by the improvements in the quality of surgical equipment and increased experience and skill of the operating surgeons, which have occurred since the original data on which we based our power calculation were published. In order to demonstrate an improvement in complete fibroid resection from 75% to 92% we would need to recruit 95 women in each arm, which is difficult to achieve in a single centre. It could be argued that a potential improvement in the success of TCRF with the use of GnRH that is less than what we expected could still be clinically significant. However, despite the small sample size of our study, the result indicates that preoperative administration of GnRH analogues is unlikely to be helpful.

The treatment and placebo groups in our study were unbalanced in terms of age as the placebo group contained no woman under 35 whereas the treatment group contained a cluster of 8 women under 35 of which 3 were under 30. However the groups were balanced in terms of parity, fibroid size and percentage of protrusion, which should confirm the absence of selection bias. Age alone is unlikely to have influenced either main or secondary outcomes.

The results of our multivariate logistic regression analysis confirmed that, of the variables analyzed, only fibroid size and degree of protrusion are significant predictors of completeness of resection.

There were no differences in the success of TCRF when the analysis was limited to those women who underwent the planned surgery. The length of the procedure, fluid deficit and complication rates were similar between the treatment and placebo groups and they were also broadly in agreement with data from the literature (Perino et al. 1993). At the follow up visit six weeks after surgery more women in the treatment group reported improvement in their symptoms, but the result was not statistically significant. This difference could be explained by a prolonged effect of GnRH analogues, which could still be present at six weeks after surgery. Long term follow up showed lower number of secondary procedures in the treatment group, but again the result was not statistically significant.

Several authors have suggested that GnRH analogue administration preoperatively may facilitate in the resection of fibroids by shrinking the tumour and thus allowing a larger proportion of the tumour to protrude into the endometrial cavity (Donnez et al. 1990; Mencaglia et al. 1993; Emanuel et al. 1997). Our results did not support this suggestion as we found no large difference in the proportion of fibroids completely resected between treatment and control group. We also could not detect a reduction in the operative interval or fluid deficit after the administration of GnRH analogue. Similarly Campo et al. (Campo et al. 2005) in a retrospective study, found that GnRH analogues are associated with prolonged operative time which they attribute to the longer time needed for cervical dilatation. In contrast Perino et al. (Perino et al. 1993) in a controlled study of 53 patients found that preoperative GnRH reduced operative time and the volume of distension medium used.

However it is unclear from the published paper whether the treatment and control groups were balanced in terms of the morphological characteristics of the fibroids submitted to TCRF. Moreover, they did not include women with fibroids over 3 cm in diameter which is the group of women that we would expect to derive the maximum benefit from preoperative GnRH.

Another rationale for the administration of preoperative GnRH analogues is a reduction of fluid deficit. Emanuel et al. (Emanuel et al. 1997) performed a retrospective study of factors that may influence fluid deficit at TCRF. They found that preoperative administration of GnRH is associated with reduced fluid deficit. However, the criteria for GnRH administration were not listed and the operating surgeon was not blinded to the treatment, which may have introduced an element of bias. Donnez et al. (Donnez et al. 1994) showed that use of GnRH reduced fibroid surface area by a third and they hypothesised that this could reduce the risk of fluid overload. In another study they found that GnRH analogues reduce fluid absorption by about 400ml (Donnez et al. 1990). Neither of these two studies were randomised or included controls. We also found a reduction in the median volume of fluid deficit in the GnRH group compared to placebo. The difference, however, was only 200 ml, which was not statistically significant. More recently a randomised controlled trial for the preoperative use of GnRH analogues was published (Muzii et al. 2010). Muzii et al in their study found that preoperative administration of GnRH analogues reduced operating time by 7 minutes and fluid absorption by 180ml both of which results were reported to be significant. Based on this they suggest that pre treatment with GnRH analogues is of benefit before TCRF. However the study suffers methodological limitations such as the operating surgeon not being blinded to the pre operative treatment. This clearly introduces operator bias and renders the endpoint

of subjective “surgeon satisfaction” unreliable. The clinical relevance of the study is also questionable as they included only Type O and Type1 fibroids less than 35mm in diameter which, as shown in this thesis, already have an excellent chance of complete resection without any pre treatment. In conclusion, they extrapolate from their results that pre treatment of women with Type 2 fibroids may be associated with a larger benefit than shown in their report for Type 1 and Type O fibroids. Our study fails to confirm such as benefit.

It could be argued that there are other potential benefits of preoperative treatment with GnRH analogues apart from facilitating a complete excision of fibroids. A meta-analysis of randomised controlled trials comparing GnRH analogue administration before abdominal myomectomy showed that women in the treatment group had significantly higher pre – operative haemoglobin concentration compared to the control group (Lethaby et al. 2001). Similar benefits are also likely to occur in women scheduled for hysteroscopic surgery.

In conclusion, our study did not demonstrate a significant benefit of administration of GnRH analogues prior to TCRF. Our results, however, are limited by a relatively small sample size and a larger multicentre trial is required to confirm our findings. Nevertheless, our trial methodology was robust and our data could be used in a meta-analysis of studies with similar outcome measures.

[4] Three dimensional power Doppler in the diagnosis of endometrial cancer

Our study found that a combination of endometrial thickness and VFI has a low false positive rate for the prediction of endometrial malignancy in high risk postmenopausal women. The high positive likelihood ratio of the test is clinically useful as patients with these characteristics will need to have their histological diagnosis expedited. However it would not be possible to use this test to discharge patients from further investigation as the negative likelihood ratio is not high enough and 1 in 10 patients so discharged would have endometrial cancer. The combination of endometrial thickness and VFI performed better than any ultrasonic variable alone significantly improving the specificity of the examination.

In contrast to our study Alcazar et al. (Alcazar et al. 2009) found that VI alone is the best predictor of malignancy with an area under the curve of 0.902. Our study did not confirm those findings as in our hands the single best predictor of malignancy was endometrial thickness. In our study none of the three vascularity indices exceeded an AUC of 0.850 which is considered necessary for a good diagnostic test. It is possible that the differences in diagnostic performance are due to differences in the study population as Alcazar et al. reported a relatively high prevalence of endometrial cancer in their sample (44.1%) and did not include endometrial hyperplasia. Our sample included 3 women diagnosed with endometrial hyperplasia with atypia on the basis that in this population such a finding would usually prompt surgical treatment in view of the high risk of malignant transformation (Horn

et al. 2007). It is interesting to note that whilst median endometrial thickness and volume in women with endometrial cancer is broadly similar between the two studies (17.7 vs. 16.3mm, 9.8 vs. 8.7 cm³) there was wide variation between the studies when it comes to the vascularity indices. Odeh et al. (Odeh et al. 2007) also performed a study employing 3D – PDA for the diagnosis of endometrial cancer reported AUCs that were lower than our study for all three vascularity indices. Again however the population studied was different to ours as they included women with postmenopausal bleeding and perimenopausal bleeding irrespective of endometrial thickness. Because of their inclusion criteria the prevalence of endometrial cancer in their study population was low (7.6%) which may explain the lower AUCs reported for all variables.

As discussed in the introduction of this thesis both machine settings, such as gain and PFR, and distance from the region of interest will influence the vascularity indices (Raine-Fenning et al. 2008; Raine-Fenning et al. 2008). Nevertheless Raine – Fenning et al. (Raine-Fenning et al. 2004) and others (Merce et al. 2006; Opolskiene et al. 2010) have shown that the reliability and reproducibility of 3D - PDA is good once the machine settings are kept constant. So the differences between studies in the quantification of vascularity indices are likely to be due to differences in machine settings and examination technique. Addressing such questions by consensus would allow studies that can be applicable across populations to be performed.

Opolskiene et al. performed a study with similar objective as the one here, investigating the usefulness of 3D – PDA in improving the positive predictive value of ultrasound in women with postmenopausal bleeding and thick endometria (Opolskiene et al. 2010). They reported similar vascularity indices for benign and malignant endometria (VI 1.4 vs. 4.2, VFI 0.5 vs.

1.3, FI 25.4 vs. 34.5) to the ones in this thesis (VI 0.7 vs. 3.1, VFI 0.2 vs. 1.1, FI 25.9 vs. 33.2). The general trend for higher values in their study is likely to be due to the difference in the power Doppler gain setting between the studies (4 vs. -0.2 in this study). In agreement with our results they found that a logistic regression model including VFI and endometrial thickness had an AUC of 0.86 with an improvement in specificity over endometrial thickness alone. These results validate the results presented in this thesis as they demonstrate that 3D – PDA examination is reproducible across populations and in different centres.

In conclusion this study has shown that none of the 3D – PDA indices alone is superior to endometrial thickness alone to determine whether an endometrium harbours a malignancy or not. At best, 3D – PDA quantification of endometrial vascularity in combination with endometrial thickness can reduce the false positive rate of the test and identify women at higher risk. However the low sensitivity of the examination does not allow women who test negative to avoid endometrial sampling which was the hypothesis of our study. Standardisation of power Doppler settings and technique is required so that any future studies on 3D – PDA examination of the endometrium will produce results that can be directly compared to our findings.

[5] Three dimensional ultrasound for the prediction of need for adjuvant therapy in endometrial cancer

Our study showed that in women with endometrial cancer measurement of the tumour volume in combination with the preoperative histological grade may be useful to assess the probability of a woman having disease Stage II or above and therefore meeting our criteria for post-operative adjuvant radiotherapy. The measurement of endometrial volume was successfully performed in all cases. The procedure adds very little time to the standard examination as the acquisition of 3D ultrasound volumes is usually completed in less than 60 seconds. The volume calculations are performed off-line and they typically take two to five minutes.

We confirmed that the tumour grade on endometrial sampling is a sensitive test for identification of women with advanced disease. However, the specificity was low and the calculated likelihood ratios indicated that tumour grade alone cannot identify accurately those women with advanced endometrial cancer stage. Endometrial thickness and endometrial volume also performed poorly when used as single predictors for additional treatment. By combining tumour grade with endometrial volume we were able to identify women that met our criteria for postoperative adjuvant therapy with a high sensitivity and specificity. The negative likelihood ratio was very low, which indicates that the test is very useful in

excluding the need for additional treatment. The positive results were also accurate but in two women the disease was less advanced than predicted by the algorithm.

The value of 2D ultrasound in the preoperative evaluation of women with endometrial cancer has been studied before. Studies of 2D transvaginal ultrasound have shown a sensitivity between 50% and 84% for the detection of myometrial invasion (Shipley et al. 1992; Kim et al. 1995). In a meta - analysis of 47 studies, ultrasound was found to be equivalent to MRI and CT for the estimation of myometrial invasion (Kinkel et al. 1999). In this study we evaluated a variety of variables, alone and in combination, to predict the stage of endometrial cancer and ultimately the need for postoperative adjuvant therapy, as dictated by the unit's criteria.

Measurement of endometrial volume by 3D ultrasound has been investigated before for its value in the diagnosis of endometrial cancer in women with postmenopausal bleeding (Gruboeck et al. 1996; Yaman et al. 2003; Merce et al. 2006; Mansour et al. 2007; Opolskiene et al. 2010). Both Merce et al and Opolskiene et al found that 3D ultrasound is reproducible for endometrial volume measurement. However the latter authors did not find that endometrial volume determination was superior to 2D endometrial thickness measurement for the diagnosis of endometrial cancer. The role of 3D ultrasound in women in whom the diagnosis of endometrial cancer has already been established is less well investigated. Alcazar et al used the virtual organ navigation modality of VOCAL to assess both objectively and subjectively the depth of myometrial invasion (Alcazar et al. 2009). They found that using a cut – off of 9mm for tumour – free distance to serosa they could achieve 100% negative predictive value with a 50% positive predictive value to diagnose more than 50% myometrial invasion. This is superior to the performance of 2D ultrasound

alone (Savelli et al. 2012). To date there are no other studies investigating the role of endometrial volume determination in preoperative endometrial cancer stage assesment (Alcazar et al. 2011). We found that both endometrial thickness and endometrial volume show a weak correlation with the stage of endometrial cancer but this on its own is unlikely to have diagnostic value. However a combination of endometrial volume measurement and histological grade improved the sensitivity and specificity of the test to determine which women had advanced disease. Despite the improved performance there would still be 2 false negative results and thus this test cannot be used to exclude women from complete surgical staging Adding further ultrasonic variables, such as the tumour – free distance, to an endometrial volume and tumour grade model may improve 3D ultrasound based preoperative staging to a point where it can be reliably used to guide the surgical approach.

Reliable information about the likelihood of women with endometrial cancer requiring additional treatment after surgery could help to modify the surgical approach depending of on the risk of women having advanced disease. It has been shown that lymphadenectomy is associated with an increased rate of intra-operative complications and a higher risk of side-effects with post-operative radiotherapy (Creutzberg 2004; Kitchener et al. 2009). In the absence of proven therapeutic benefits the routine use of lymphadenectomy in all patients is questionable as it may not be required in women with clear evidence of advanced disease, who require post-operative radiotherapy (Kitchener et al. 2009). By using information about preoperative tumour grade and volume, those women with low risk of advanced disease could be offered a standard staging laparotomy with pelvic nodal dissection. Women at high risk of advanced disease could be spared of pelvic node clearance as they would require adjuvant radiotherapy as well. Furthermore, these patients could be selected for a thorough para-aortic

lymph node dissection with the rationale that positivity could justify extension of the radiotherapy fields. The full pelvic lymph node dissection in the women predicted to be at low risk of radiotherapy would enable the identification of a small proportion of women with occult advanced disease who would otherwise not have received radiotherapy. Admittedly, this group would be exposed to both lymphadenectomy and pelvic radiotherapy.

Our model was accurate in identifying women with endometrial cancer Stage less than II and they could be selected for a full staging laparotomy with confidence. The result indicating the need for adjuvant treatment was also reliable. Nevertheless, two women with localised disease would not have had full pelvic nodes sampling as they had been classified as having advanced disease. In our population the rate of Grade III tumours was relatively high as was the proportion of women with advanced disease. The study was carried out at a tertiary centre and this may have resulted in selection bias.

In conclusion, we present our results, not with the intention of stipulating the surgical and adjuvant management of endometrial cancer, but with the aim of highlighting a potentially useful algorithm using clinical, ultrasound and histological data to guide surgical management and ultimately, adjuvant treatment. We appreciate that the utility of adjuvant radiotherapy in the management of endometrial cancer remains a controversial area and continues to be the subject of randomised controlled studies. However, our methods could potentially be improved by the inclusion of other ultrasound or MRI derived criteria such as myometrial invasion and the presence of enlarged lymph nodes. Ultimately, any algorithm would need to be prospectively tested before it could be used in routine clinical practice.

Part V Conclusions & Further Research

This thesis has explored some aspects of the diagnosis and treatment of uterine pathology, fibroids and endometrial cancer, with a focus on the role three – dimensional ultrasound and associated modalities can play to improve diagnostic accuracy.

The thesis has shown that 3D – SIS can be used effectively to select patients with submucous fibroids for transcervical resection. We found that a fibroid's intramural depth and size are good predictors of fibroid resectability and should guide clinician's decision making. The question that remains unanswered is whether the classification system proposed is valid. This can only be addressed with prospective validation. 3D – SIS is currently the only method for preoperative assessment of submucous fibroids for which the reproducibility has been demonstrated. This thesis has demonstrated that 3D – SIS provides objective information which can be used to accurately predict the outcome of surgery over and above what can be derived from a diagnostic hysteroscopy. This is due to the ability of 3D – SIS to obtain global views of the uterus and provide accurate reproducible measurements. Considering the additional benefits of a digital dataset that can be manipulated to obtain the best views and can be stored indefinitely to be compared with subsequent examinations, 3D – SIS in the opinion of this author should take the place of diagnostic hysteroscopy as the gold standard examination for the assessment of submucous fibroids.

In terms of submucous fibroid treatment our randomized controlled trial did not find a benefit in treating patients selected for TCRF with GnRH analogues preoperatively. This study however was limited by our sample size and may have not been sufficiently powered to detect a small effect of GnRH analogues. In any case such a small effect (if present) may not be clinically significant or may not justify exposing patients to significant side effects that are associated with GnRH analogues. This trial attempted to fill a gap in the evidence base that

guides the management of submucous fibroids. Almost simultaneously with the completion and publication of our trial another study was published (Muzii et al. 2010). However this trial did not include women with type II fibroids that are speculated to derive the maximum benefit from preoperative GnRH treatment. Similarly to our results that trial did not demonstrate a large clinical improvement to justify exposing women to added morbidity. The results presented in this thesis suggest that routine use of GnRH analogues before TCRF is not warranted. To explore smaller scale benefits than our sample size allows, our results could form part of a meta-analysis as our methodology and randomisation process was sound.

In this thesis we presented a study that challenged the orthodox view of fibroid development, describing fibroids that shrink despite a stable hormonal environment. Despite the widespread nature of fibroids, to date there are only 3 published longitudinal scientific studies on fibroid development including the one that was produced as a result of this thesis. Our results exposed our incomplete understanding of the pathophysiology of these tumours and showed that fibroids are a heterogeneous group that do not grow in a uniform way. Support for these findings comes from an MRI based longitudinal study that reported similar heterogeneity in fibroid growth patterns (Peddada et al. 2008). Our study suffered methodological limitations as it was retrospective which limited the ability to control for the influence of time in the menstrual cycle and race on fibroid development. Fibroids are a significant cause of morbidity for women and further study is needed.

Our studies of endometrial cancer also revealed areas where three – dimensional ultrasound can improve diagnostic accuracy. 3D – PDA showed promise as an adjunct to endometrial thickness as an initial assessment tool. Based on our results, the finding of high VFI in bleeding postmenopausal women and a thick endometrium should alert clinicians to a higher

risk of malignancy. However 3D – PDA did not achieve the aim of being a reliable second line test as its sensitivity was not adequate as a basis for clinical decisions. On their own 3D – PDA indices do not appear to provide information over and above endometrial thickness. Whether 3D – PDA will find a role as an adjunct to other ultrasonic variables remains to be seen but to achieve this aim the examination parameters both in terms of technique and machine settings need to be standardised. While the interobserver variability for 3D – PDA acquisition and analysis have been shown to be good it is also clear that the vascularity indices are sensitive to machine settings alterations and the distance from the region of interest. Researchers should reach a consensus as to the appropriate settings so that future research in different centres can be comparable and could be combined in a systematic review (Alcazar et al. 2011).

The study presented here on the use of 3D ultrasound to measure endometrial volume for endometrial cancer staging is novel. To date 3D applications in this field have focussed on the assessment of women with postmenopausal bleeding without a clear benefit shown for endometrial volume over endometrial thickness. We have shown that both variables correlate with endometrial cancer stage but this association is not strong enough to make firm predictions. Addition of histological grade in a logistic regression improved the diagnostic performance but still did not achieve a level which could be clinically useful. Others have shown that detailed 2D transvaginal ultrasound or 3D ultrasound virtual organ navigation, have promise for the appreciation of myometrial invasion (Savelli et al. 2008; Alcazar et al. 2009). Clearly endometrial volume may become another weapon in the armamentarium of the clinician and can provide useful information combined with other ultrasonic variables for the preoperative staging of endometrial cancer.

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Appendix

This thesis has resulted in the following publications:

- Mavrellos, D., Ben – Nagi J., Holland T., Hoo W., Naftalin J., Jurkovic D. (2010). "The natural history of fibroids." *Ultrasound Obstet Gynecol* 35(2): 238-42.
- Mavrellos, D., Ben – Nagi J., Davies A., Lee C., Salim R., Jurkovic D. (2010). "The value of preoperative treatment with GnRH analogues in women with submucous fibroids: a double-blind, placebo-controlled randomized trial." *Hum Reprod* 25(9): 2264-9.
- Mavrellos, D., Nafatlin J., HooW., Ben – Nagi J., Holland T., Jurkovic D. (2011) "The preoperative assessment of submucous fibroids by three – dimensional saline infusion sonohysterography." *Ultrasound Obstet Gynecol.* 38(3):350 - 4